A CRITICAL REVIEW

BURN AND RAND'S HYPOTHESIS

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I. INTRODUCTION

The most outstanding advancement in the physio-pharmacology of the autonomic nervous system, during the last decade, has been in the basic mechanism involved in the release of the neurotransmitter at the postganglionic sympathetic nerve endings. The classical theory of neurohumoral transmission as proposed by Elliot (58) has so far faced the test of time very elegantly. The all-round acceptability of Elliot's concept can well be imagined by the fact that even today most of our understanding about the physiological systems and the mechanism of action of the pharmacologically active substances has been based on the neurohumoral mechanisms operating at various synapses. Not only this, the similarities between the action of numerous drugs and the effects observed by stimulation of different areas of the brain, have led to the important discovery of the humoral nature of impulse transmission in different areas of the central nervous system.

In the year 1934, Dale (50) classified the postganglionic fibres of the entire autonomic nervous system as either 'adrenergic' or 'cholinergic' depending upon the neurohumor involved in transmission of impulses from the nerve endings to the effector organ. Besides the identification of choline in the suprarenal extract by Hunt and Taveau (81) and postulation of acetylcholine (Ach) by Otto Loewi (96) as the neurohumor of the parasympathetic system, identification of noradrenaline (NA) by Eular (59) at the sympathetic postganglionic nerve endings was a unique advancement in attributing the two naturally occurring substances viz. Ach and NA as the physiological neurotransmitters of the two anatomical divisions of the autonomic nervous system.

For about a decade the things appeared settled. But in the year 1958 and in the following years Burn and Rand (33, 35) boldly put forward their hypothesis regarding the role of Ach in the release of NA from the postganglionic sympathetic nerve endings. It appeared for a while that Ach, which used to be classified as the neurohumor perpetuating parasympathetic activity, is also creeping in the dominion of NA i.e. the postganglionic sympathetic nerve endings. Soon the physiologists and pharmacologists became conscious in interpreting the results of any study on the postganglionic fibres of the autonomic nervous system. Burn and Rand subsequently published a series of papers in support of their hypothesis.

The credit for demonstrating the presence of nerve fibres liberating Ach in the region of the postganglionic sympathetic nerve endings should not be exclusively given to Burn and Rand. As early as 1934 Dale (50) wrote "we can then say that postganglionic parasympathetic fibres are predominantly and perhaps entirely 'cholinergic' and that postganglionic sympathetic fibres are predominantly though not entirely, 'adrenergic' while some, and probably all, of the preganglionic fibres of the whole autonomic system are 'cholinergic,". Further more there had been a number of reports regarding the presence of cholinergic fibres in the anatomically classified postganglionic sympathetic nerves, e.g. Bacq and Fredericq (7) suggested the presence of cholinergic fibres in the postganglionic nerves supplying the nictitating membrane of cat; Sherif (115) put forward the evidence for the presence of cholinergic fibres in the sympathetic supply of the dog uterus; Folkow *et al.* (66) showed the presence of cholinergic fibres running

from the stellate ganglion to the heart. Not only these, detailed pharmacological studies of the sympathetic outflow to the hind paw and foot of the cat (76, 65), coronary vessels of the cat (66), buccal cavity and lips of the dog (60) and sweat glands of the cat (51) have suggested that the cholinergic fibres are responsible for some of the paradoxical results obtained upon sympathetic nerve stimulation. Presence of cholineacetylase and cholinesterase, the two enzymes concerned in the synthesis and metabolism respectively of Ach, in adrenergic neurones as reported by Koelle (86) itself suggests some role of Ach in sympathetic fibres. All the more Ach contents of the blood vessels have been shown to be decreased after sympathetic denervation (4). Similarly excision of the superior cervical ganglion in rabbit results in a definite reduction of the acetylcholinesterase in the innervation of the nictitating membrane (82). However, none of the earlier workers suggested any physiological significance of these cholinergic fibres in the mediation of adrenergic response in these tissues. But certainly Burn and Rand deserve credit for putting up their hypothesis concerning the role of Ach in the release of adrenergic neurotransmitter substance at the postganglionic nerve endings.

During the last five years or so, other workers have reported a number of experimental observations which are not explainable on the basis of the theory propounded by Burn and Rand. All the more the opponents of this hypothesis have proposed alternative explanations for the experimental results obtained by Burn and Rand group of workers. On the other hand the proponents of this hypothesis and their followers might have imagined a stage ahead probably, that not only electrical stimulation of the sympathetic postganglionic fibres but also drugs releasing catecholamines from the stores might be doing so through the release of Ach. In view of the variable results of similar studies and varied interpretations of comparable results by different workers each one of these experimental results requires separate reconsideration in the light of our present day knowledge about the physiology, anatomy, biochemistry and pharmacology of the postganglionic nerve endings. As such it is intended in this review to summarize the various observations and their interpretations by various workers which either favour or disprove the existence of a cholinergic link in adrenergic mediation.

II. BURN AND RAND'S HYPOTHESIS

"Burn and Rand (33, 35) postulated that all the postganglionic sympathetic fibres have cholinergic mechanism involved in the release of noradrenaline after nerve stimulation i.e. postganglionic sympathetic nerve stimulation first releases Ach which triggers off the mechanism by which NA is liberated from the local tissue stores at the sympathetic postganglionic nerve endings".

Burn and Rand's hypothesis is singularly striking in that it involves a cholinergic link in adrenergic mediation in contrast to earlier belief that nerve stimulation directly releases NA to produce an adrenergic response. This would imply that the whole of the autonomic nervous system is basically cholinergic. Whatever be the actual status of Burn and Rand's hypothesis, if Ach liberates catecholamines from the stores one might imagine that there could be three possibilities: (*a*) the postganglionic fibres might release only Ach which would in turn release NA from the adjacent chromaffin cells, a mechanism similar to that operating in adrenal

medulla; (*b*) the release of Ach by the postganglionic impulse might initiate or facilitate the liberation of NA from the same fibre; (*c*) the release of Ach from the postganglionic fibres might initiate or facilitate the liberation of NA from different nerve fibres. The last possibility would mean that in the postganglionic sympathetic nerves there are separate cholinergic and adrenergic fibres and the former regulate the liberation of neurohumor from the latter fibres.

From whatever site Ach might be liberating the catecholamines, the actual mechanism of the adrenergic neurohumoral release at the sympathetic postganglionic nerve endings is far from clear. Douglas and Rubin (56) had shown that Ach released catecholamines from the adrenal medulla through the agency of calcium ions. This was evident by the fact that Ach, in absence of calcium ions, failed to release catecholamines from the isolated perfused adrenal medulla. Further more the amount of catecholamine release was related to the concentration of the calcium ions in the perfusion fluid. These workers have further postulated that Ach evoked secretion of catecholamines by promoting calcium influx into the chromaffin cells (57). Burn and Gibbons (41) have recently suggested that the situation is similar at the sympathetic postganglionic terminations. They observed that the inhibition, produced in rabbit ileum by stimulating the periarterial nerves present in the mesentary or by nicotine in presence of hyoscine, was proportional to the concentration of the calcium ions present in the bath. These observations suggest that calcium ions are, at least, essential for the release of catecholamines by Ach from the sympathetic postganglionic nerve endings.

III. EVIDENCES IN SUPPORT OF BURN AND RAND'S HYPOTHESIS

A. *Pharmacological Studies*

(a) *Studies on the tufts of hairs on cat's tail* :—

Chronologically the earliest observation which seems to support Burn and Rand's hypothesis was that of Brucke (23) in the year 1935. He observed that small dose (5 μg) of Ach injected at the base of a tuft of hairs in cat's tail produced piloerection, a response comparable to that observed on stimulation of the sympathetic fibres. This piloerection was followed by blockade of the response to nerve stimulation. Similar results have been described with Ach and nicotine by Coon and Rothman (49). These workers further demonstrated that prior degeneration of the postganglionic nerves abolished this pilomotor response to Ach and nicotine. This clearly suggests that intact sympathetic innervation is essential for this pilomotor response. Such a concept seems to be correct in view of the observations of Burn *et al.* (30) that even after pharmacological denervation i.e. reserpinization, both nicotine and small dose of Ach were ineffective to produce piloerection. Again this ineffectiveness has been attributed to lack of catecholamines in the stores. Such a view has been supported by their observation that treatment with reserpine greatly reduced the amount of NA, which could be extracted from the skin of the cat's tail, and it also reduced the number of chromaffin cells and their granules. Studies carried out by Burn and Rand (36) with hemicholinium, which is known to block the synthesis of Ach (99, 98), are most conclusive in support of their hypothesis. They observed that the erection of the tufts of hairs to repeated sympathetic stimulation was slower in onset if hemicholinium had been injected at their basis. All the more these tufts were relaxed

at a time when untreated tufts were still erect. These workers concluded that nicotine and small doses of Ach liberate NA to produce piloerection, a response which is markedly inhibited after blockade of the synthesis of Ach by hemicholinium and totally absent after depletion of catecholamines from the stores produced either by degeneration of nerves or by reserpinization. Recently Rand and Whaler (110) have reported that botulinum toxin, which blocks the release of Ach from the cholinergic nerves (25), if injected into the skin of cat's tail at the base of a tuft of hairs, prevents piloerection to sympathetic stimulation. This indicates that release of Ach following sympathetic nerve stimulation is essential for piloerection.

When large dose of Ach was injected at the base of the tuft of hairs, Burn and Rand observed a transitory piloerection followed by blockade of the response to sympathetic nerve stimulation. The explanation put forward by them for such an observation was that large doses of Ach blocked the release of NA from local catecholamine stores.

(b) *Studies on the nictitating membrane* :—

Bacq and Fredericq (7) were the first to describe the presence of cholinergic fibres in the innervation of the nictitating membrane of the cat. Stimulation of the nerve supplying the nictitating membrane is well known to produce contractile response. Burn and Rand (35) observed that even in reserpine pretreated animals nerve stimulation contracts the nictitating membrane. This response in reserpinized preparations was found to be potentiated by eserine and blocked by atropine suggesting that stimulation of the postganglionic sympathetic fibres released Ach like substance. Similar results have been reported by Burn *et al.* (38) using isolated nictitating membrane preparation. However, the results obtained by other workers with anticholinesterase agents and cholinergic blocking agents are variable. These could probably be because of the differences in the experimental methods and their interpretation of the observations. However, the results obtained by Burn *et al.* (38, 32) and Browman *et al.* (22) have been suggested to support Burn and Rand's hypothesis. The failure on the part of physostigmine to increase the responses of the nictitating membrane to postganglionic stimulation in the studies of Gardiner *et al.* (69) has been explained by Burn and Rand (37) on the grounds that they did not perfuse the head and did not give atropine or hyoscine first. In fact Gardiner *et al.* (69) administered physostigmine by putting it into the conjunctival sac so that the amount of the drug reaching the blood stream was not known.

Nicotine produces contraction of the nictitating membrane of cat. After reserpinization or nerve degeneration this response of the nictitating membrane to nicotine has been reported to be reduced (30). This could be due to lack of catecholamines susceptible to release by nicotine. Such a concept has been supported by the fact that both these procedures i.e. reserpinization and nerve degeneration reduce the number of chromaffin cells and their granules (30).

Jacobowitz *et al.* (83) studied the effect of hemicholinium on the neuronally induced contractions of the nictitating membrane in rabbits. They observed that hemicholinium produced a considerable reversible reduction in the isometric contraction of the nictitating membrane to supramaximal tetanic stimulation of the sympathetic postganglionic trunk. This was well accounted by the inhibitory effect of hemicholinium on the synthesis of Ach leading to gradual exhaustion of the stores of Ach.

Burn *et al.* (32) studied the effect of hyoscine on the contractions of the nictitating membrane to nerve stimulation with different frequencies. They reported that hyoscine produced a significant reduction in the amplitude of contractions of the membrane during supramaximal postganglionic stimulation at lower frequencies (below 5 shocks/sec) but at higher frequencies, when the response was maximal, little or no reduction was observed. These workers suggested that the maximal response obtained with higher frequencies (i.e. hyoscine resistant component which was probably adrenergic in nature) might be masking the hyoscine sensitive (cholinergic) component. Similar were the observations of Nystrom (107) from the extracellular recording of the smooth muscle action potentials in the cat nictitating membrane. He observed that single preganglionic shock produced two negative potentials, the primary phase could be blocked by hyoscine (cholinergic in nature) and the secondary (hyoscine resistant) was blocked by phenoxybenzamine and piperoxan. All the more the hyoscine sensitive component was potentiated by physostigmine.

Burn *et al.* (32) showed that when the postganglionic fibres from the superior cervical ganglion were stimulated supramaximally, the contraction of the membrane increased in size as the frequency rose. In the presence of hyoscine, the contractions of the membrane were smaller; the greatest difference being for the lowest stimulus frequency. This difference decreased as the frequency increased. They have suggested that there is release of Ach to act directly only at lower frequencies of stimulation and that at higher frequencies, Ach was almost entirely used to release NA. Such a concept is supported by similar observations of Burn *et al.* (38) that in the nictitating membrane of hyoscine or atropine treated cat, where the response to Ach should have been blocked, anticholinesterase agents like neostigmine produced an increase in the height of contractions of supramaximal postganglionic stimulation at frequencies below 5 shocks/sec. This potentiation was found to be maximal at lowest frequencies.

Ambache (3) showed that botulinum toxin, which blocks the release of Ach from the cholinergic nerves (25), reduced the responses of nictitating membrane to sympathetic stimulation. It can be inferred that Ach was contributing directly or indirectly through NA release in the responses of the nictitating membrane to sympathetic stimulation.

(c) *Studies on intestine and colon* :—

Gillespie and Mackenna (72, 73) observed that in reserpinized animal, electrical stimulation of the sympathetic fibres produced contraction of the colon. This stimulant effect was blocked by atropine. Studies with hemicholinium on this preparation by Burn and Rand (36) revealed that hemicholinium reduced the inhibitory effect of sympathetic nerve stimulation on the isolated colon of untreated animals. It can be inferred that decreased availability of Ach after hemicholinium was responsible for the reduced adrenergic response. All the more this effect of hemicholinium was reversed by choline.

Studies on the isolated rabbit ileum with mesentary attached by Burn *et al.* (32) suggested that stimulation of periarterial nerves released both Ach and NA depending upon the frequency of stimulation. At lower frequencies both Ach and NA were released while at higher frequencies only NA was released. The evidence in favour of this concept is that hyoscine in-

creased the inhibitory effect of nerve stimulation on the ileum when it was stimulated with lower frequencies but was ineffective when higher frequencies of nerve stimulation were employed. Recently Rand and Whaler (110) found that botulinum toxin added to the isolated organ bath blocked the inhibitory effect of nerve stimulation on rabbit ileum. This has been suggested to be due to blockade of the release of Ach from the nerve endings by botulinum toxin.

(d) *Studies on the vas deferens* :—

The contractions of the isolated guineapig vas deferens, a preparation previously thought to possess only sympathetic innervations, to electrical stimulation have been reported to be potentiated by Ach (119) and anticholinesterase agents like physostigmine, neostigmine (19, 29, 108, 112), tetramethoquin and demecarium (14). These observations clearly suggest that increased availability of Ach either by exogenous source or by inhibition of cholinesterase enzyme potentiates these responses. Studies by Chang and Rand (47) with hemicholinium showed that it decreased the contractions of the isolated guineapig vas deferens preparation to nerve stimulation. When the nerve stimulation was ineffective to produce any response addition of Ach to the bath produced contraction and this stimulant effect of Ach was found to be blocked by atropine. Recently Rand and Whaler (110) have reported that botulinum toxin which blocks the release of Ach (25) abolished the contractions of the vas deferens to sympathetic nerve stimulation. This would mean that release of Ach is essential in the mediation of nerve impulses in this preparation.

(e) *Studies on the uterus* :—

Sherif (115) reported that the sympathetic supply to the uterus in dog possessed cholinergic fibres also. Burn and Rand (35) reported that stimulation of the hypogastric nerve in reserpinized cats produced contraction of the uterus in contrast to the inhibitory effect observed in normal cats. This stimulant effect, observed in reserpinized cats, was found to be potentiated after inhibition of the cholinesterase enzyme (by eserine) and was blocked by atropine. These observations suggest that the excitatory effect following postganglionic nerve stimulation in reserpinized cats was probably due to release of Ach.

(f) *Studies on the spleen* :—

The ability of adrenaline to cause contraction of spleen *in vivo* has been reported in numerous investigations (1, 75, 79, 109). Bradon and Rand (21) found that degeneration of the spleenic nerve resulted in loss of Ach as well as of catecholamine contents of the spleen. Burn and Rand (36) observed that stimulation of the postganglionic fibres produced either an increase or a decrease in the volume of the spleen obtained from reserpine pretreated animals. In cases where there was an increase in the volume of the spleen, eserine potentiated and atropine blocked this effect suggesting that dilatation of spleen in these cases was probably due to release of Ach. In cases where contraction of spleen was observed, addition of eserine was without any effect while atropine converted the increase into decrease in the volume of spleen. These workers suggested that in these cases reserpine had not completely depleted the catecholamines from the stores and as such after blockade of the cholinergic receptors by atropine, catecholamines released after stimulation

of the postganglionic fibres produced contraction of the spleen. Release of Ach following postganglionic nerve stimulation was amply confirmed when these workers (21) observed that the perfusate from the spleen of reserpinized cats stimulated the isolated guineapig ileum. This stimulant effect of the perfusate on the guineapig ileum was found to be blocked by atropine (21). These observations clearly indicate that nerve stimulation released Ach. Further more this release of Ach-like substance was found to be increased in presence of an anticholinesterase agent (Neostigmine).

The sympathomimetic effects of Ach on the spleen previously described by Farber (61) have been re-investigated by other workers in dog (52) and cat (20, 21). They observed that close intra-arterial injection of Ach into the spleen produced contraction of spleen and this effect of Ach could be prevented by pretreatment with reserpine or dibenzyline or hexamethonium and also by degeneration of the sympathetic supply. Ferry (63) has suggested that Ach produces sympathomimetic effects on the spleen by excitation of the postganglionic adrenergic fibres. Ach in presence of atropine produces an effect on the spleen like that of sympathetic stimulation. This effect of Ach was blocked by bretylium and hexamethonium. Blockade by bretylium would indicate that Ach-induced release of catecholamines was blocked by this compound. Hexamethonium has been suggested to block the action of Ach at the sympathetic nerve endings by preventing its entry into the sympathetic fibres (42).

Studies with hemicholinium carried out by Bradon and Rand (21) showed that it blocked the contractions of the spleen induced by sympathetic nerve stimulation. This blockade has been reported to be reversed by choline. These workers further reported that hemicholinium also blocked the vasoconstriction in the perfused spleen following nerve stimulation. Even this effect was found to be partially reversed by choline (21). It implies that once the Ach contents of the preparation have been exhausted, nerve stimulation is ineffective to produce any sympathomimetic effect.

Electron microscopic studies have revealed that the terminations of each sympathetic postganglionic fibre of the rat spleen contain two kinds of vasicles, some contain dense granules and the others are empty (homogenous vasicle) (54). After reserpine pretreatment, the granular vasicles disappear. Autoradiography combined with electron microscopy has demonstrated that the granular vasicles store exogenous (H^3) NA. The granular vasicles were found to be closely similar to the synaptic vasicles of the central nervous system and the motor end plate. These studies suggest that nerve terminals possess vasicles for both the neurohumors i.e. Ach and NA.

(g) *Studies on the cardiac tissues* :—

Another earlier observation favouring Burn and Rand's hypothesis was that of Hoffman *et al.* (77) who showed that in isolated perfused and atropinized heart of various species like cat, rabbit and guineapig, Ach produced positive inotropic and chronotropic effects resembling those observed after sympathetic stimulation. They felt that this stimulant action of Ach, in presence of atropine, was mediated through the release of a substance which had the characteristics of catecholamine. Confirmatory evidence for such a concept was obtained

when they identified an adrenaline like substance in the perfusate with Ach. All the more sympathetic denervation of the heart and treatment with reserpine reduced or abolished the effect of Ach (2, 8, 43). The obvious explanation of these observations would be that Ach released NA from the stores of the sympathetic postganglionic nerve endings of the heart. The intactness of sym pathetic postganglionic fibers has been recently reported to be essential for Ach to have its full effects (44). All the more Hukovic (80) showed that 2:6 xylyl ether and bretylium blocked Ach-induced positive inotropic effect on isolated rabbit atria in presence of atropine. The obvious explanation would be that these two drugs block the release of catecholamines susceptible to Ach.

Pharmacological response, similar to that observed with Ach, has been reported with nicotine on the heart by various workers. Nicotine produces a biphasic response i.e. an initial negative followed by positive inotropic and chronotropic response on isolated heart or atria of cat, rabbit and guineapig (74, 88, 93, 103, 124). Similar effects have also been reported with nicotine on isolated rat atria (48). Kottegoda (88) found that nicotine, in atropinized perfused rabbit atria, increased the force and rate of atrial contractions. These effects were found to be blocked by hexamethonium, a ganglion blocking agent. As such, at that time, the effects were attributed to the ganglionic actions of nicotine. Contrary to this concept Lee and Shideman (94) showed that nicotine produced similar effect on cat papillary muscle, a preparation which is devoid of ganglia. As such the response observed by Kottegoda (88) could not be attributed to the ganglionic effects of nicotine. A satisfactory explanation about the mechanism of these actions of nicotine came with the work of Burn and Rand (33). They showed that procedures like pharmacological denervation i.e. reserpinization as well as surgical denervation, which led to the depletion of catecholamines from the nerve endings of the rabbit atria, rendered the tissues insensitive or less sensitive to the stimulant action of nicotine. Finally in uninnervated embryonic chick heart both Ach and nicotine produced positive inotropic effects. Extractable amounts of catecholamines have been reported in uninnervated embryonic chick heart (95). All these observations seem to suggest that both Ach and nicotine stimulate the atropinized heart through the release of catecholamines from the local tissue stores.

Release of Ach after stimulation of the sympathetic fibres of the isolated rabbit atria has been suggested (36). Stimulation of these sympathetic fibres supplying the atria obtained from normal rabbits produced positive chronotropic effect. On the contrary, in atria, obtained from reserpinized rabbits, a negative inotropic effect has been reported (36). In addition this inhibitory effect could be potentiated by eserine and blocked by atropine (36). In isolated cat atria Day and Rand (53) showed that after guanethidine, stimulation of postganglionic fibres caused cardio-inhibitory effect. In the same preparation obtained from normal cats Leaders (90) showed that stimulation of the sympathetic nerves produced classical cardio-stimulant effect which was followed by a small but consistent cardio-inhibitory effect. This inhibitory component was potentiated by physostigmine and blocked by atropine. All the more this response could be blocked by hemicholinium both in normal and reserpinized preparations.

All these observations indicate that sympathetic nerve stimulation releases Ach which in turn regulates the release of NA from the stores. However, the mechanism of noradrenaline

release by Ach is not yet clear. It could do so by exciting the postganglionic sympathetic fibres at some point so that impulses spread throughout the ramifications of the fibres and release NA in a normal way (49). Alternately it might release NA from the sympathetic fibres without initiating impulses in the fibres (37). If the former explanation holds good, the impulses initiated peripherally would be expected to be conducted antidromically back towards the cell bodies in the sympathetic ganglia. Coon and Rothman (49) suggested that Ach injected into the skin sets up propagated impulses to produce piloerection at a distance of several centimetres from the point of injection. Recently Cabrera *et al.* (44) have suggested that Ach sets up antidromic impulses in the postganglionic sympathetic fibres and these must at least contribute to the sympathomimetic effects of Ach on the heart.

(h) *Studies on blood vessels* :—

(*i*) Ear vessels:—Burn and Dutta (39) and Kottegoda (89) studied the effects of Ach and nicotine on rabbit's ear vessels. They reported that Ach (in presence of atropine) and nicotine produced vasoconstriction. This vasoconstriction was reversed after tolazoline, an alpha adrenergic receptor blocking agent. This reversal has been suggested to be due to precipitation of beta receptor effects of catecholamines released by Ach and nicotine. These observations have been confirmed by Burn and Rand (34). These workers have further reported that Ach in presence of atropine and nicotine fails to produce vasoconstriction in rabbit ear vessels after reserpine pretreatment or nerve degeneration. All the more this vasoconstrictor effect has been reported to be blocked by bretylium and 2:6 xylyl ether (80) which block the release of NA from the storage sites.

Release of Ach, essential for the liberation of NA after stimulation of sympathetic postganglionic nerve endings, has also been suggested in rabbit ear vessels. Stimulation of the postganglionic nerves has been long known to produce vasoconstriction in these vessels. Studies on the intact rabbit ear by Holton and Rand (78) have shown that stimulation of postganglionic sympathetic fibres produces a decrease in blood content, which has been attributed by them to vasoconstriction, followed by an increase in blood content which indicates vasodilatation. This vasodilatation was enhanced by eserine and blocked by atropine. Even the vasoconstrictor effect following postganglionic sympathetic nerve stimulation in isolated rabbit ear vessels has been reported to be potentiated by eserine. However, high doses of Ach blocked this vasoconstrictor effect to sympathetic stimulation (35). The explanation put forward by these workers is that large doses of Ach block the release of NA following nerve stimulation. Studies with hemicholinium reported by Burn and Rand (36) indicate that it blocked the vasoconstrictor effect of nerve stimulation on rabbit ear vessels. Farther more this effect of hemicholinium was found to be reversed by choline. All these observations support the view that sympathetic postganglionic nerve stimulation releases Ach which in turn liberates NA from the stores to produce sympathomimetic effects. Confirmatory evidence for such a mechanism has been found from the work of Burn and Rand (36) when they detected the presence of a substance in the perfusate which had a stimulant effect on the leech muscle.

(*ii*) Hind limb vessels : The earliest observation suggesting a cholinergic phenomenon in dog's hind limb was that of Burn (27) in the year 1932. He observed that stimulation of the sympathetic nerve supply of hind leg in dog produced vasodilatation in some experiments and vasoconstriction in others. Three years later Bulbring and Burn (24) demonstrated that some fibres to the vessels of the hind leg of the dog were cholinergic in nature. After the usual vasoconstrictor response to nerve stimulation had been blocked by guanethidine, sympathetic nerve stimulation produced vasodilatation (28, 102). This vasodilatation was blocked by atropine. Recently Bernard and Schaepdryver (11) have shown that in atropinized dog anticholinesterase agents potentiate the vasoconstriction which occurs in the dog hind leg upon electrical stimulation of the nerve.

(*iii*) Accessory cephalic vein : Rice and Long (111) observed that high doses of Ach injected into the perfused accessory cephalic vein of the dog produced vasoconstriction. This response has been suggested to be due to release of catecholamines from the extraneuronal storage sites as this response was inhibited by pretreatment with reserpine or phentolamine and was potentiated by cocaine. All the more the venous effluent after Ach could relax the isolated chick rectal caecum and this relaxant effect was blocked by pronethalol, an adrenergic beta receptor blocking agent. These observations indicate that there is release of catecholamines by Ach in this preparation. The possibility of a ganglionic site of action has been excluded by the fact that this effect was not blocked by hexamethonium.

(*iv*) Systemic blood pressure studies : Tyramine, an agent well known for its catecholamine releasing action, has been reported to produce fall in blood pressure in reserpinized animals (46, 101, 125). This depressor response to tyramine has been attributed to be due to release of Ach as it is potentiated by eserine and blocked by atropine (46). Studies by these workers on isolated atria and intestine of rabbit also favour the view that tyramine releases Ach. It could be that Ach, released by tyramine, might be responsible for its catecholamine releasing action. Release of catecholamines has also been suggested after choline. Singh (117) reported that hexamethonium and atropine converted the usual depressor response of choline in rat to pressor. This pressor response was blocked by bretylium and dibenzyline and hence this pressor response has been attributed to be due to NA release. Release of catecholamine by choline has also been suggested by the studies of Srimal *et al.* (121).

(i) *Studies on central nervous system* :—

The solitary report which seems to support Burn and Rand's hypothesis in adrenergic mediation in the central nervous system is that of Srimal *et al.* (121). They reported that intracerebroventricular injection of choline in anaesthetized dogs produced biphasic response on the blood pressure level. This response consisted of an initial hypertensive followed by delayed hypotensive phase. The initial hypertensive phase has been attributed to be due to choline perse as it is blocked by atropine. The delayed hypotensive effect was a prolonged one resembling that observed after intracerebroventricular NA. This hypotensive phase was abolished by prior reserpinization of the animal and blocked by prenethalol and INPEA, the two adrenergic beta receptor blocking agents. This blockade wac similar to that reported by Bhargava

et al. (13) for intracerebroventricular NA. All the more there was tachyphylaxis to this hypotensive phase. These workers concluded that intracerebroventricular choline caused vasodepressor response due to release of catecholamines probably in the region of medullary vasomotor centre.

Studies with hemicholinium on central vasoregulatory mechanisms by Sinha *et al.* (118) indicate that it depressed the medullary vasomotor centre. They are of the opinion that Ach is intimately concerned in the regulation of vasomotor tone. Hemicholinium has been reported to inhibit synthesis of Ach in the brain also (67). It could be that inhibition of Ach synthesis might depress the adrenergic mediation in these neurones.

B. *Histochemical Studies*

Presence of acetylcholinesterase has been demonstrated in the sympathetic ganglia in various species (71, 84, 85,) as well as in the muscle portions of the vas deferens, nictitating membrane, uterus and fallopian tube of the cat (56). The acetylcholinesterase activity of the ganglia and their perikarya has been reported to be fairly proportionate as determined by histochemical examination (86) and by ultramicroanalysis (71). If this enzyme activity of the sympathetic ganglia reflects the presence of the same enzyme in the region of the postganglionic sympathetic nerve endings then Ach could be expected of some physiological significance in sympathetic mediation as proposed by Burn and Rand. All the more, not only there was presence of acetylcholinesterase in the sympathetic ganglia, there was fairly good correlation between the concentrations of acetylcholinesterase, Ach and choline acetylase in the various regions of the central and peripheral nervous system (26, 62, 87, 97, 105, 106). Furthermore Burn and Philpot (31) showed that degeneration of postganglionic fibres to the nictitating membrane is associated with a fall in the cholinesterase activity in this tissue. Recently Jacobowitz and Koelle (82), studying the acetylcholinesterase activity of vas deferens, uterus, fallopian tube and nictitating membrane in different species, have concluded that the results are consistent with the occurrance of a cholinergic link in adrenergic mediation in the vas deferens of the guineapig and nictitating membrane of the rabbit.

IV. EVIDENCES AGAINST BURN AND RAND'S HYPOTHESIS

During the last five years or so, a number of experimental observations have been reported by various workers which are not explainable on the basis of Burn and Rand's hypothesis. All the more alternative explanations have been put forward to explain the experimental results which are said to support this hypothesis. The tissues employed by these workers, in addition to nictitating membrane and vas deferens preparations, are the Finkleman's preparation, isolated cat atria nerve preparation, spontaneously beating sinoatrial (SA) node preparation and various preparations of the blood vessels.

A. *Pharmacological Studies*

(a) *Studies on the nictitating membrane* :—

Mirkin and Cervoni (104) carried out detailed studies on the effects of various drug treatments on neuronally induced contractions of the nictitating membrane in cats. They observed

that in reserpinized animals atropine even in a dose of 12 *mg/kg* did not completely abolish the contractions of the nictitating membrane to stimulation of postganglionic fibres. On the contrary Burn and Rand (35) have reported complete block of these responses even in a dose of 4 *mg/kg* of atropine. A partial explanation put forward by Mirkin and Cervoni (104) for this discrepancy is that Burn and Rand used an isotonic lever whereas Mirkin and Cervoni used force displacement transdusor for recording the contractions of this preparation. However, one would not attribute the two entirely opposite results solely to be due to two different recording systems. Mirkin and Cervoni (104) observed that atropine in a dose of 100 $\mu g/kg$, which effectively blocked vagal induced bradycardia, depressed both neuronally and adrenaline induced contractions of the nictitating membrane pointing towards a nonspecific blocking action of atropine. Similar nonspecific antagonism by atropine on the same tissue has also been reported by other workers (45, 123). To avoid this nonspecificity of atropine, Mirkin and Cervoni used methyl atropine. Methyl atropine was found to produce insignificant depression of the development of tension following nerve stimulation both in normal and in reserpinized preparations. All the more catecholamine induced contractions of this preparation were unaffected even when it blocked the cardiovascular effects following either Ach administration or nerve stimulation.

Studies with anticholinesterase agents by Mirkin and Cervoni (104) revealed another discrepancy between their results and those reported by Burn and Rand (35). They observed that intravenous administration of physostigmine failed to alter the development of tension following nerve stimulation. Burn and Rand (35), on the other hand, reported that physostigmine decreased the stimulus threshold required to produce a given response. Potentiation of these responses by physostigmine were described by Bacq and Fredericq (6) as early as 1934. However, these observations of Bacq and Fredericq (6) and Burn and Rand (35) should not be regarded as conclusive evidences for any cholinergic transmission in view of the fact that physostigmine has been shown to potentiate the responses to exogenous catecholamines both *in vivo* (114) and *in vitro* (45).

Even studies with hemicholinium by these workers do not agree well with those of Burn and Rand (36). Mirkin and Cervoni (104) failed to observe any inhibitory effect on the contractions of the nictitating membrane to postganglionic nerve stimulation both in normal and reserpinized preparations. The results obtained by these workers are essentially in agreement with the *in vitro* studies of Gardiner and Thompson (68) and *in vivo* studies of Wilson and Long (129). These contradictory reports regarding the effects of hemicholinium on the contractions of the nictitating membrane could, as one might imagine, be due to the differences in the frequencies of stimulation because at higher frequencies of stimulation the contractions decreased almost immediately. Strikingly, hemicholinium did not alter the rate at which the contractions decayed. All the more choline did not diminish this rate of decay of these responses. As such these findings do not support the concept of a cholinergic link in adrenergic transmission.

Phentolamine, an adrenergic alpha receptor blocking agent, has been reported to block the responses of nictitating membrane to nerve stimulation by seventy per cent in rabbits (83) and by fifty per cent in cats (104). Further addition of atropine after phentolamine in rabbits failed to block the contraction of the nictitating membrane any more. All the more phentolamine did not block the nictitating membrane responses in reserpinized cats. These observations in rabbits do not support the idea of any cholinergic mediation in this preparation. Mirkin and Cervoni (104), however, observed that bretylium and xylocholine, the two adrenergic neurone blocking agents, antagonized the contractions of the nictitating membrane to postganglionic nerve stimulation in reserpinized cats. These observations are well in agreement with those of Burn and Rand (35). The explanation put forward by these workers presupposes two receptor pools, say 'A' and 'B'. Pool 'A' is accessible to both the liberated neurohumor and the antagonist while pool 'B' is available to the neurohumor only. In normal animal the antagonist i.e. phentolamine blocks the receptors of pool 'A' while in reserpinized animal pool 'A' is devoid of any neurohumor, and pool 'B' is primarily involved in elicitation of a response. As such the adrenergic blocking agents are not capable of blocking this response. Similar incapability of adrenergic blocking agents to completely antagonize the responses of sympathetic nerve stimulation was described by Nickerson and Nomaguchi (106) as early as 1948.

(*b*) *Studies on colon* :—

Studying with Finkleman's preparation, Bentley (9) observed that nerve stimulation produced a stimulant effect in preparations obtained from animals treated with reserpine or reserpine plus bretylium or reserpine plus guanethidine or with guanethidine plus eserine. Analysing this effect he suggested that such a stimulant effect could be either due to stimulation of sympathetic cholinergic fibres as postulated by von Eular and Gaddum (60) or due to a cholinergic trigger mechanism as postulated by Burn and Rand (35) or due to stimulation of parasympathetic fibres in the periarterial nerves. This stimulant effect was found to be blocked by ganglian blocking agents like hexamethonium and pempidine and it has been suggested that there are periarterial parasympathetic fibres present in this preparation. However, Day and Rand (53) failed to observe any blockade of these responses in this preparation after similar concentrations of ganglian blocking agents in the bath solution.

In contrast to reserpinization, pretreatment with bretylium alone, did not result in any contractile response to nerve stimulation in Finklemen's preparation (9, 53). One of the possible explanations could be that bretylium blocked the so called cholinergic trigger mechanism of Burn and Rand. However, this explanation cannot hold good in view of the fact that bretylium was ineffective to block the contractile response to nerve stimulation in this preparation obtained from reserpine pretreated animals. Such an observation rules out the ganglian blocking property of bretylium (70) to be involved in blocking the contractile response to nerve stimulation in this preparation.

A cholinergic trigger mechanism, if exists in this perparation, should be expected to potentiate sympathetic response after a combination of carbamyl choline and atropine or nicotine and eserine. In fact this was not so. Nicotine blocked the inhibitory responses in the intestine

to sympathetic stimulation (9). Studies with hemicholinium showed that it did not block the inhibitory response of this preparation to nerve stimulation. This would mean that any cholinergic mechanism is not likely to be involved in the inhibitory response of sympathetic nerve in this preparation.

(c) *Studies on the uas deferens* :—

Detailed pharmacological studies, carried out by Bhargava *et al.* (14) on the isolated guineapig vas deferens preparation do not support the view that there is a cholinergic link in the sympathetic responses of this preparation. They observed that atropine partially inhibited the neuronally induced contractions of this tissue. The potentiating effect of anticholinesterase agents on these responses has been attributed by Birmingham (15) to two factors, firstly there might be potentiation of the effect of Ach at the muscarinic receptors and secondly there would be inhibition of the cholinesterase enzyme in the ganglia leading to enhanced effectiveness of the preganglionic volleys in releasing noradrenaline from the postganglionic adrenergic nerve endings. The second explanation does not appear to be involved in view of the reports of Bhargava *et al.* (14) that the potentiating effect of anticholinesterase agents on this preparation was found to be blocked by atropine. The potentiating effects of phenoxybenzamine on the responses of guineapig vas deferens preparation to nerve stimulation (19, 40) have also been attributed to the anticholinesterase activity of the compound. However, the potentiation observed with anticholinesterase agents does not seem to be related to catecholamine in any way, because this potentiation was not affected by prior reserpinization of the preparation (14). Tranylcypromine, a monoamine oxidase inhibitor has been found to potentiate the responses of this tissue to nerve stimulation (14). The most interesting observation of Bhargava *et al.* (14) on this preparation was that when the responses of the vas deferens preparation to nerve stimulation were potentiated by anticholinesterase agents, furtehr potentiation of the responses was obtained with inhibitors of the enzymes concerned with the metabolism of catecholamines in the body i.e. by monoamine oxidase and catechol-*o*-methyl transferase inhibitors. All the more the reverse is also true i.e. after potentiation with monoamine oxidase and catechol-*o*-methyl transferase inhibitors further potentiation was observed by anticholinesterase compounds (14). Furthermore, in the presence of phenoxybenzamine, they observed that physostigmine potentiated the response. Similarly, in presence of atropine, adrenergic agents could potentiate the responses of the vas deferens to nerve stimulation. Phenoxybenzamine was effective in blocking noradrenaline induced potentiation of the response without interfering with that observed with physostigmine. On the basis of all these observations, these workers have concluded that cholinergic and adrenergic mechanisms operate independently of each other in this preparation.

The question regarding the presence of ganglia modifying the drug effect on this preparation has attracted the attention of many workers. Presence of ganglia in this preparation has been demonstrated both by histological methods (108) and with the use of ganglian blocking agents (10, 20). Sjostrand (120) showed that the contractions of the vas deferens elicited by hypogastric nerve stimulation were inhibited by ganglion blocking drugs like hexamethoniun, tetraethylammonium, azamethonium, lobeline and nicotine. Histologically Vogt (127) also demonstrated the presence of ganglia and chromaffin tissue in the hypogastric nerve of the dog.

Ferry (64) provided evidence that ganglia are present in close proximity of the nerve supplying the vas deferens. In view of these reports on the presence of ganglia in this preparation, the significance of the inhibitory effect of hemicholinium on the neuronally induced contractions of the isolated guineapig vas deferens preparation, as reported by Chang and Rand (47), fades away. This blockade could well be due to the ganglionic site of action of hemicholinium and it seems that Chang and Rand (47) were stimulating the preganglionic fibres. As such hemicholinium, a post-synaptic blocking agent (100, 122) would be expected to block these sites. Furthermore, the potentiating effect of physostigmine in presence of atropine could be due to its ganglionic action resulting in release of NA from the postganglionic nerve endings. This seems to be the likely mechanism because in reserpine pretreated preparations physostigmine was ineffective in overcoming the block produced by atropine.

To exclude the possibility of any ganglionic action of hemicholinium in this preparation Bentley and Sabine (10) studied the effect of hemicholinium on the contractions of the vas deferens to transmural stimulation. The responses of the vas deferens obtained by this method have been attributed to the stimulation of postganglionic nerve fibres and part of these fibres are cholinergic postganglionic fibres (16). Bentley and Sabine (10) observed that hemicholinium produced partial block (less than 50%) of these responses. The development of this partial block was gradual as compared to the complete block of the neuronally induced contractions. It could be that hemicholinium like hexamethonium produces most of its blocking action on the hypogastric nerve-vas deferens preparation by blocking ganglian cells and not by an action on the sympathetic nerve endings as suggested by Chang and Rand (47). Such an explanation is supported by the observation of Macintosh *et al.* (99) that hemicholinium caused failure of transmission of impulses in the superior cervical ganglian of the cat. If hemicholinium exerts its blocking action exclusively through its ganglionic effects, it may explain the failure to observe any blockade of the responses of the rabbit colon (9) and cat nictitating membrane (68) to nerve stimulation.

(d) *Studies on spleen :—*

Leaders and Dayrit (92) studying the effect of hemicholinium on the isolated perfused spleen observed that hemicholinium did not alter the responses of the spleen to nerve stimulation. Furthermore, they could not detect any Ach like substance in the effluent as opposed to the identification of an Ach like substance in the effluent by Bradon and Rand (21). After hemicholinium Ach still produced its characteristic response indicating that muscarinic receptors were not blocked. However, at this stage nicotine was ineffective to produce any response. This, together with the failure to detect any Ach like substance in the effluent, indicates that cholinergic transmission was blocked by hemicholinium. The fact, that the spleen still responded to nerve stimulation after the cholinergic mechanism (at the ganglia) had been inhibited, suggests that there is separate adrenergic component in the spleen rather than any intermediary cholinergic link.

Blakeley *et al.* (17) observed that inhibition of the cholinesterase enzyme by eserine or prostigmine in doses large enough to affect profoundly the skeletal neuromuscular transmission

did not alter NA output suggesting that preservation of Ach at the nerve endings failed to increase the release of NA from local catecholamine stores. The sympathomimetic effects of Ach on the spleen have been suggested by Bradon and Rand (21) as evidence to support the idea of a cholinergic link in the postganglionic pathway. Blakeley *et al.* (17) feel that Ach owes its effects due to excitation of motor fibres, leading in turn to liberation of NA and consequent contraction of spleen. The liberation of NA by Ach was abolished by hexamethonium in doses that leave quite unaffected the motor response of the spleen when its nerves were stimulated. The liberation and uptake of NA by nerve impulses were similarly not diminished by hexamethonium, in doses sufficient to suppress the effects of injected Ach. The sympathomimetic effects of injected Ach on the spleen can not, therefore, be regarded as evidence for a cholinergic link in the sympathetic postganglionic pathways (17).

(e) *Studies on cardiac tissue* :—

(*i*) Isolated cat atria-nerve preparation: Leaders (90) studied the effect of hemicholinium and nicotine on the isolated cat atria preparation. He observed that hemicholinium did not inhibit the response of this preparation to sympathetic nerve stimulation, nor did it block the response to nicotine administration. Had there been any cholinergic link the response to nerve stimulation should have been blocked by hemicholinium.

(*ii*) Spontaneously beating sinoatrial node preparation : Vincenzi and West (126) studied the problem of a cholinergic link in adrenergic mediation with the help of isolated spontaneously beating sinoatrial node preparation. Direct electrical stimulation of this preparation elicits a biphasic chronotropic response (5, 128). This biphasic response comprises a primary negative chronotropic effect, which is cholinergically mediated as it is potentiated by physostigmine and blocked by atropine, and a secondary positive chronotropic effect, which is adrenergically mediated as it is potentiated by cocaine and is abolished by pretreatment with reserpine, guanethidine and bretylium and blocked by dichloroisoproterinol. In such a preparation Vincenzi and West (126) found that the cholinergically mediated negative chronotropic response was effectively blocked by hemicholinium. On the contrary, the positive chronotropic response was found to be resistant to even ten times the concentration of hemicholinium required to block the negative chronotropic effect. These observations rule out the possibility that there might be any cholinergic link in the mediation of adrenergic response in this preparation.

(f) *Studies on blood vessls* :—

(*i*) Ear vessels: Studies by Rogers and Leaders (113) on the isolated perfused rabbit ear vessels do not suggest the presence of any cholinergic link in the mediation of vasoconstriction to nerve stimulation. These workers did not observe any secondary vasodilatation following postganglionic nerve stimulation supplying the ear essels. Even after reserpinization, a vasodilator response was not observed by them. Studies with nicotine by these workers on the same preparation suggest the ganglionic site of action in nicotine response. They feel that the lack of a cholinergic component in the response observed with nicotine is an added evidence against the cholinergic link in adrenergic mediation or of separate cholinergic fibres.

(*ii*) Intestinal blood vessels : Studying the perfused isolated mesenteric artery Boatman and Brody (18) observed that intra-arterial injection of Ach produced a biphasic response on perfusion pressure. There was an initial increase followed by decrease in perfusion pressure. They observed that the intitial increase in perfusion pressure following Ach was not due to catecholamine release as it is not reduced by reserpinization but was abolished by atropine. They concluded that the stimulant action of Ach on the muscle fibres was responsible for increasing the perfusion pressure.

(*iii*) Hind limb vessels : Leaders (91) studying the effect of hemicholinium on the isolated perfused hind leg of the dog showed that it did not abolish the vasoconstriction to repeated sympathetic nerve stimulation or to injected Ach or nicotine. This would mean that blockade of synthesis of Ach by hemicholinium does not interfere with the mediation of adrenergic response. However, hemicholinium blocked the vasodilatation to stimulation of sympathetic nerves in "acutely reserpinized" animals. He concluded that there are separate cholinergic and adrenergic components in the sympathetic innervation of the vascular bed of the hind limb of the dog rather than a cholinergic junction in the adrenergic fibres as proposed by Burn and Rand.

(*iv*) Pulmonary vessels : The neurohumoral mechanism operating in the isolated sympathetic nerve pulmonary artery preparation of the rabbit, has recently been studied by Bevan and Su (12). They observed that even high concentrations of Ach and hemicholinium did not produce any effect on the contractile response of these vessels to sympathetic nerve stimulation. Yohimbine, bretylium and reserpine blocked completely the response to nerve stimulation. They have concluded that transmission of impulses in this preparation at the sympathetic postganglionic nerve endings is mediated by an adrenaline like transmitter substance and provide no evidence for the view that Ach is at all involved in the vasoconstriction to sympathetic nerve stimulation in the pulmonary artery preparation.

(*v*) Skeletal muscle vessels : Dorr and Brody (55) carried out studies on the postganglionic sympathetic nerves leading to isolated perfused gracillis muscle. They showed that in this preparation, hemicholinium was ineffective in blocking the sympathetic cholinergic fibres supplying the blood vessels. The ability to maintain the adrenergic but not the cholinergic responses indicates that liberation of the adrenergic transmitter could not be dependent on the release of an intermediate cholinergic transmitter as proposed by Burn and Rand using other tissues.

B. *Histochemical Studies*

Lack of enzymes involved in the synthesis and metabolism of any neurohumor would oppose the idea of any physiological role of the neurohumor in synaptic transmission. Gardiner *et al.* (69) reported that the nictitating membrane of the cat is virtually devoid of staining for acetylcholinesterase, the enzyme concerned in the metabolism of Ach. Jacobowitz and Koelle (82) reported that the acetylcholinesterase staining in the vas deferens and nictitating membrane of cat was much more restricted as compared to catecholamine fluorescence in the same tissues.

These reports do not seem to support the widespread physiological role of Ach in the mediation of sympathetic impulses in these tissues.

V. CONCLUDING REMARKS

At the present stage the exact status of Burn and Rand's hypothesis is far from definite. We have experimental observations both in favour and against the presence of a cholinergic link in sympathetic mediation. In view of the cholinomimetic responses of the tissues to electrical stimulation of the so called postganglionic sympathetic nerves three possibilities are worth consideration. (1) Firstly, these fibres could probably be preganglionic and there might be sympathetic ganglionic synapses in these tissues, anatomically similar to the ganglionic synapses of the parasympathetic system. As such, the cholinergic response obtained in these studies could be due to the diffusion of Ach from these ganglionic synapses. (2) Secondly, in addition to the adrenergic fibres, these anatomically classified postganglionic sympathetic nerves also contain separate cholinergic fibres which might be responsible for the cholinomimetic responses of these tissues. (3) Thirdly, all these fibres might be basically cholinergic in nature, but the release of NA might be dependent on the cholinergic link as proposed by Burn and Rand. Whatever be the source of Ach in the region of the postganglionic sympathetic nerve ending, its involvement in the release of NA after nerve stimulation is not yet convincing. Even studies with hemicholinium and botulinum toxin, which could be expected to clear off the doubts about this theory, are not univocal. From the point of view of evolution it might be that cholinergic mechanism of neurohumoral transmission is primitive and adrenergic mechanism has been gradually superimposed in order to cope with the environmental changes during critical periods. As such, at the present stage, we have a mixture of cholinergic and adrenergic innervation. If this is true, observation of cholinergic responses of the tissues after nerve stimulation should actually not be regarded as evidence for a cholinergic link in the release of NA at the sympathetic nerve endings. However, we will have to wait till conclusive evidences, either in favour or against this hypothesis, are available. It may be that this hypothesis may have to face difficult time.

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REFERENCES

1. Ahlquist, R.P., J.P. Taylor, C.W. Rawson and V.L. Sydow. Comparative effects of epinephrine and levarterenol in the intact anesthetized dog. *J. Pharmacol. Exp. Therap.* **110**: 352, 1954.
2. Alvarado, F., S. Middleton and J.P. Beca. Efecto de la reserpina y de la adrenalectomia sobre la accion cardioestimulante de la acetilcolina. *Acta, Physiol. Latinoam.* **11**: 236, 1961.
3. Ambache, N. Unmasking, after cholinergic paralysis by botulinum toxin of a reversed action of nicotine on the mammalian intestine revealing the possible presence of local inhibitory ganglion cells in the enteric plexus. *Brit. J. Pharmacol*, 6:51, 1951.

4. Armin, J., R.T. Grant, R.H.S. Thompson and A. Tickner. An explanation for the hightened vascular reactivity of denervated rabbit's ear. *J. Physiol.* **121**:603, 1953.

5. Armory, D.W. and T.C. West. Chronotropic response following direct electrical stimulation of the isolated sinoatrial node, a pharmacological evaluation. *J. Pharmacol. Exp. Therap.* **137**:14, 1962.

6. Bacq, Z.M. and H. Fredericq. L'innervation sympathique postganglionnaire de la membrane nictitante du chat pent etre en partie de nature cholinergique. *C.R. Soc. Biol. Belg.* **117**:482, 1934.

7. Bacq, Z.M. and H. Fredericq. Essai d'identification du mediateur chimique libere dans la membrane nictitante du chat par l' excitation sympathique. *Arch. Int. Physiol.* **40**:297, 1935.

8. Benitez, D. Mecanismo del efecto cardioestimulante de la acetilcolina. *Thesis, University of Chile*, 1957.

9. Bentley, G.A. Studies on sympathetic mechanisms in isolated intestinal and vas deference preparations. *Brit. J. Pharmacol.* **19**:85, 1962.

10. Bentley, G.A. and J.R. Sabine. The effects of ganglion blocking and postganglionic sympatholytic drugs on preparations of guinea pig vas deferens. *Brit. J. Pharmacol.* **21**: 190, 1963.

11. Bernard, P.J. and A.F. De Schaepdryver. Adrenergic mechanism in the dog hind leg. *Arch. Int. Pharmacodyn.* **148**:301, 1964.

12. Bevan, J.A. and C. Su. The sympathetic mechanism in the isolated pulmonary artery of the rabbit. *Brit. J. Pharmacol.* **22**:176, 1964.

13. Bhargava, K.P., B.P. Jaju and K.K. Tangri. Mechanism of central hypotensive action of guanethidine. *Brit. J. Pharmacol.* **27**:491, 1966.

14. Bhargava, K.P., K. Kar and Surendra S. Parmar. Independent cholinergic and adrenergic mechanisms in the guineapig isolated nerve vas deferens preparation. *Brit. J. Pharmacol.* **24**:641, 1965.

15. Birmingham, A.T. The potentiation by anticholinesterase drugs of the responses of the guineapig isolated vas deferens to alternate preganglionic and postganglionic stimulation. *Brit. J. Pharmacol.* **27**:145, 1966.

16. Birmingham, A.T. and A.B. Wilson. Preganglionic and postganglionic stimulation on the guineapig isolated vas deferens preparation. *Brit. J. Pharmacol.* **21**:569, 1963.

17. Blakeley, A.G.H., G.L. Brown and G.B. Ferry. Pharmacological experiments on the release of the sympathetic transmitter. *J. Physiol.* **167**:505, 1963.

18. Boatman, D.L. and M.J. Brody. Effects of acetylcholine on the intestinal vasculature of the dog. *J. Pharmacol. Exp. Therap.* **142**:185, 1963.

19. Boyd, H., Y. Chang and M.J. Rand. The anticholinesterase activity of some antiadrenaline agents. *Brit. J. Pharmacol.* **15**:525, 1960.

20. Bradon, K.W. and H. Boyd. Release of noradrenaline from the spleen of cat by acetylcholine. *Nature.* **192**:880, 1961.

21. Bradon, K.W. and M.J. Rand. Acetylcholine and sympathetic innervation of the spleen. *J. Physiol.* **157**:18, 1961.

22. Browman, W.C., B.A. Callingham and A.W. Cuthbert. The effect of physostigmine on the mechanical and electrical responses of the cat nictitating membrane. *Brit. J. Pharmacol.* **22**:558, 1964.

23. Brucke, F.T. Uber die wirkung von acetylcholin auf die pilomotoren. *Klin. Wochschr.* **14**:7, 1935.

24. Bulbring, E. and J.H. Burn. The sympathetic dilator fibres in the muscles of the cat and dog. *J. Physiol.* **83**:483, 1935.

25. Burgen, A.S.V., F. Dickens and L.J. Zatman. The action of botulinum toxin on the neuromuscular transmission. *J. Physiol.* **109**:10, 1949.

26. Burgen, A.S.V. and L.M. Chipman. Cholinesterase and succinic dehydrogenase in the central nervous system of the dog. *J. Physiol.* **114**:296, 1951.

27. Burn, J.H. On vasodilator fibres in the sympathetic and on the effect of circulating adrenaline in augmenting the vascular response to sympathetic stimulation. *J. Physiol.* **75**: 144, 1932.

28. Burn, J.H. A new view of adrenergic nerve fibres explaining the action of reserpine, bretylium and guanethidine. *Brit. Med. J.* **1**:1623, 1961.

29. Burn, J.H. and D.F. Weetman. The effect of eserine on the response of the vas deferens to hypogastric nerve stimulation. *Brit. J. Pharmacol.* **20**:74, 1963.

30. Burn, J.H., E.H. Leach, M.J. Rand and J.W. Thompson. Peripheral effects of nicotine and acetylcholine resembling those of sympathetic stimulation. *J. Physiol.* **148**:332, 1959.

31. Burn, J.H. and F.J. Philpot. Effect of sympathetic denervation on the cholinesterase in the nictitating membrane and the iris. *Brit. J. Pharmacol.* **8**:248, 1953.

32. Burn, J.H., J.J. Dromy and B.J. Large. The release of acetylcholine by sympathetic nerve stimulation at different frequencies. *Brit. J. Pharmacol.* **21**:97, 1963.

33. Burn, J.H. and M.J. Rand. Action of nicotine on the heart *Brit. Med. J.* **1**:137, 1958.

34. Burn, J.H. and M.J. Rand. Noradrenaline in the arterial walls and its dispersal by reserpine. *Brit. Med. J.* **1**:903, 1958.

35. Burn, J.H. and M.J. Rand. Sympathetic postganglionic cholinergic fibres. *Brit. J. Pharmacol.* **15**:56, 1960.

36. Burn, J.H. and M.J. Rand. A new interpretation of the adrenergic nerve fiber, in Advances in Pharmacology. Ed. S. Garattini and P.A. Shore. **1**:1. Academic Press, New York, 1962.

37. Burn, J.H. and M.J. Rand. Acetylcholine in adrenergic transmission. *Ann. Rev. Pharmacol.* **5**:163, 1965.

38. Burn, J.H., M.J. Rand and R. Wien. The adrenergic mechanism in the nictitating membrane. *Brit. J. Pharmacol.* **20**:83, 1963.

39. Burn, J.H. and N.K. Dutta. The action of antagonists of acetylcholine on the vessels of rabbit's ear. *Brit. J. Pharmacol.* **3**:354, 1948.

40. Burn, J.H. and W.R. Gibbons. The effect of phenoxybenzamine and of tolazoline on the response to sympathetic stimulation. *Brit. J. Pharmacol.* **22**:527, 1964.

41. Burn, J.H. and W.R. Gibbons. The part played by calcium in determining the response to stimulation of sympathetic postganglionic fibres. *Brit. J. Pharmacol.* **22**:540, 1964.

42. Burn, J.H. and W.R. Gibbons. The sympathetic postganglionic fibre and the block by bretylium; the block prevented by hexamethonium and limited by mecamylamine. *Brit. J. Pharmacol.* **22**:549, 1964.

43. Cabrera, R., A. Cohen, S. Middleton, L. Utano and H. Viveros. The immediate source of noradrenaline released in the heart by acetylcholine. *Brit. J. Pharmacol.* **27**:46, 1966.

44. Cabrera, R., R.W. Torrance and H. Viveros. The action of acetylcholine and other drugs upon the terminal parts of the postganglionic sympathetic fibres. *Brit. J. Pharmacol.* **27**: 51, 1966.

45. Cervoni, P., T.C. West and L.D. Fink. Autonomic postganglionic innervation of the nictitating membrane of the cat. *J. Pharmacol. Exp. Therap.* **116**:90, 1956.

46. Chandra, O., K.N. Dhawan and G.P. Gupta. Release of acetylcholine by tyramine. *Arch. int. Pharmacodyn.* **157**:141, 1965.

47. Chang, V. and M.J. Rand. Transmission failure in sympathetic nerve produced by hemicholinium. *Brit. J. Pharmacol.* **15**:588, 1960.

48. Chiang, T.S. and F.E. Leaders. Mechanism for nicotine and DMPP on the isolated rat atria-vagus nerve preparation. *J. Pharmacol. Exp. Therap.* **149**:225, 1965.

49. Coon, J.M. and S. Rothman. The nature of the pilomotor response to acetylcholine; some observations on the pharmacodynamics of the skin. *J. Pharmacol. Exp. Therap.* **68**:301, 1940.

50. Dale, H.H. Nomenclature of fibres in the autonomic system and their effects. *J. Physiol.* **80**: p. 10, 1934.

51. Dale, H.H. and W. Feldberg. The chemical transmission of secretory impulses to the sweat glands of the cat. *J. Physiol.* **82**:121, 1935.

52. Daly, M. De B. and M.J. Scott. The effect of acetylcholine on the volume and vascular resistance of the dog's spleen. *J. Physiol.* **156**:246, 1961.

53. Day, M.D. and M.J. Rand. The effect of guanethidine in revealing cholinergic sympathetic fibres. *Brit. J. Pharmacol.* **17**:245, 1961.

54. De Robertis, E. and A. Pellegrino de Iraldi. A plurivesicular component in adrenergic nerve endings. *Anat. Record.* **139**:299, 1961.

55. Dorr, L.D. and M.J. Brody. Functional separation of sympathetic adrenergic anticholinergic fibres. *Fed. Proc.* **23**:540, 1964.

56. Douglas, W.W. and R.P. Rubin. The role of calcium in the secretory response of the adrenal medulla to acetylcholine. *J. Physiol.* **159**:40, 1961.

57. Douglas, W.W. and R.P. Rubin. Mechanism of catecholamine release from adrenal medulla and the role of calcium in stimulus-secretion coupling. *J. Physiol.* **167**:288, 1963.

58. Elliot, T.R. On the action of adrenaline. *J. Physiol.* **31**: p. xx, 1904.

59. Eular, U.S. von. A specific sympathomimetic ergone in adrenergic nerve fibres; sympathin and its relations to adrenaline and noradrenaline. *Acta, Physiol. Scand.* **12**:73, 1946.

60. Eular, U.S. von. and J.H. Gaddum. Pseudomotor contractures after degeneration of the facial nerve. *J. Physiol.* **73**:54, 1931.

61. Farber, S. The action of acetylcholine on the volume of the spleen of the dog. *Arch. int. Pharmacodyn.* **53**:367, 1936.

62. Feldberg, W. and M. Vogt. Acetylcholine synthesis in different regions of the central nervous system. *J. Physiol.* **107**:372, 1948.

63. Ferry, C.B. The sympathomimetic effect of acetylcholine on the spleen of cat. *J. Physiol.* **167**:487 p, 1963.

64. Ferry, C.B. The postganglionic fibres of the vas deferens of the guineapig. *J. Physiol.* **169**:72 p, 1963.

65. Folkow, B. and B. Uvnas : Distribution and functional significance of sympathetic vasodilators to hind limb of cat. *Acta, Physiol. Scand.* **15**:389, 1948.

66. Folkow, B., J. Frost, K. Haeger and B. Uvnas. Cholinergic fibres in sympathetic outflow to heart in dog and cat. *Acta, Physiol. Scand.* **15**:421, 1948.

67. Gardiner, J.E. The inhibition of acetylcholine synthesis in brain by a hemicholinium. *Biochem. J.* **81**:297, 1961.

68. Gardiner, J.E. and J.W. Thompson Lack of evidence of a cholinergic mechanism in sympathetic transmission. *Nature.* **191**:86, 1961.

69. Gardiner, J.E., K. Hellmann and J.W. Thompson. The nature of the innervation of the smooth muscle, harderian gland and blood vessels of the cat's nictitating membrane. *J. Physiol.* **163**:436, 1962.

70. Gertner, S.B. and A. Romano. Action of guanethidine and bretylium on ganglionic transmission. *Fed. Proc.* **20**:319, 1961.

71. Giacobini, E. The distribution and localization of cholinesterase in nerve cells. *Acta, Physiol. Scand.* **45**, Supple. **156**:1, 1959.

72. Gillespie, J.S. and B.R. Mackenna. The effect of reserpine on the response of the rabbit ileum and colon to stimulation of their extrinsic nerves *in vitro*. *J. Physiol.* **147**: p 31, 1959.

73. Gillespie, J.S. and B.R. Mackenna. The inhibitory action of the sympathetic nerves of the smooth muscle of the rabbit gut, its reversal by reserpine and restoration by catecholamines and dopa. *J. Physiol.* **156**:17, 1961.

74. Giotti, A. Interaction of nicotine and eserine, ephedrine, atropine, hexamethonium and adrenaline in isolated guineapig auricles. *Brit. J. Pharmacol.* **9**:15, 1954.

75. Grindley, J.H., J.F. Herrick and F.C. Mann. Measurement of the blood flow of the spleen. *Amer. J. Physiol.* **127**:106, 1939.

76. Hinsey, J.C. and C.C. Cutting. The sherrington phenomenon. *Amer. J. Physiol.* **105**:535, 1933.

77. Hoffman, F., E.J. Hoffman, S. Middleton and J. Talesnik. The stimulating effect of acetylcholine on the mammalian heart and the liberation of an epinephrine like substance by isolated heart. *Amer. J. Physiol.* **144**:189, 1945.

78. Holton, P. and M.J. Rand. Sympathetic vasodilatation in rabbit ear. *Brit. J. Pharmacol.* **19**:513, 1962.

79. Holtz, P., F. Bachmann, A. Engelhardt and K. Greeff. Die Milzwirkung des adrenalins und arterenols. *Pflugers. Arch. Ges. Physiol.* **255**:232, 1952.

80. Hukovic, S. The action of sympathetic blocking agents on isolated and innervated atria and vessels. *Brit. J. Pharmacol.* **15**:117, 1960.

81. Hunt, R. and R. De M. Taveau. On the physiological action of certain choline derivatives and new method for detecting choline. *Brit. Med. J.* **2**:1788, 1906.

82. Jacobowitz, D. and G.B. Koelle. Histochemical correlation of acetylcholinesterase and catecholamines in the postganglionic autonomic nerves of the cat, rabbit and guineapig. *J. Pharmacol. Exp. Therap.* **148**:225, 1965.

83. Jacobowitz, D., P. Johnson, I. Kitchner and G.B. Koelle. The effect of hemicholinium (HC-3) on sympathetic transmission at the nictitating membrane of the rabbit. *Brit. J. Pharmacol.* **25**:527, 1965.

84. Koelle, G.B. Cholinesterases of the tissues and the sera of rabbits. *Biochem. J.* **53**:217, 1953.

85. Koelle, G.B. The histochemical localization of cholinesterases in the central nervous system of the rat. *J. Comp. Neurol.* **100**:211, 1954.

86. Koelle, G.B. The histochemical identification of acetylcholinesterase in cholinergic, adrenergic and sensory neurones. *J. Pharmacol. Exp. Therap.* **114**:167, 1955.

87. Koelle, G.B. Heffter-Haubner Hand b and Exp. Pharm. Suppl. **15**:187. Springer and Verlay, Heidelberg, 1963.

88. Kottegoda, S.R. Stimulation of isolated rabbit auricles by substances which stimulate ganglia. *Brit. J. Pharmacol.* **8**:83, 1953.

89. Kottegoda, S.R. The action of nicotine and acetylcholine on the vessels of rabbit ear. *Brit. J. Pharmacol.* **8**:156, 1953.

90. Leaders, F.E. Local cholinergic adrenergic interaction; mechanism for the biphasic chronotropic response to nerve stimulation. *J. Pharmacol. Exp. Therap.* **142**:31, 1963.

91. Leaders, F.E. Separation of adrenergic and cholinergic fibres in sympathetic nerves to the hind limb of the dog by hemicholinium. *J. Pharmacol. Exp. Therap.* **148**:238, 1965.

92. Leaders, F.E. and C. Dayrit. The cholinergic component in the sympathetic innervation of the spleen. *J. Pharmacol. Exp. Therap.* **147**:145, 1965.

93. Leaders, F.E. and J.P. Long. Mechanism of the positive chronotropic response to nicotine. *J. Pharmacol. Exp. Therap.* **137**:206, 1962.

94. Lee, W.C. and F.E. Shideman. Role of myocardial catecholamines in cardiac contractility. *Science.* **129**:967, 1959.

95. Lee, W.C., L.P. McCarty, W.W. Zodrow and F.E. Shideman. The cardiostimulant action of certain ganglionic stimulants on the embryonic chick heart. *J. Pharmacol. Exp. Therap.* **130**:30, 1960.

96. Loewi, O. Ueber humorale uebertragbarkeit der Herznervenwirhung (ii Mitteilung) *Pflugers. Arch. Ges. Physiol.* **193**:201, 1921.

97. MacIntosh, F.C. The distribution of acetylcholine in the peripheral and the central nervous system. *J. Physiol.* **99**:436, 1941.

98. MacIntosh, F.C. Effect of HC-3 on acetylcholine turnover. *Fed. Proc.* **20**:562, 1961.

99. MacIntosh, F.C., R.I. Birks and P.B. Sastry. Pharmacological inhibition of acetylcholine synthesis. *Nature.* **178**:1181, 1956.

100. Martin, A.R. and R.K. Orkand. Postsynaptic effects of HC-3 at the neuromuscular junction of the frog. *Canad. J. Biochem. Physiol.* **39**:343, 1961.

101. Maxwell, R.A., H. Povalski and A.T. Plummer. A differential effect of reserpine on pressor amine activity and its relationship to other agents producing this effect. *J. Pharmacol. Exp. Therap.* **125**:178, 1959.

102. McCubbin, J.W., Y. Kaneke and I.H. Page. The peripheral cardiovascular actions of guanethidine in dogs. *J. Pharmacol. Exp. Therap.* **131**:346, 1961.

103. McDowall, R.J.S. Stimulating action of acetylcholine on heart. *J. Physiol.* **104**:392, 1946.

104. Mirkin, B.L. and P. Cervoni. The adrenergic nature of transmission in the cat nictitating membrane following treatment with reserpine. *J. Pharmacol. Exp. Therap.* **138**:301, 1962.

105. Nachmansohn, D. Chemical and molecular basis of nerve activity. Academic Press, New York, 1959.

106. Nickerson, M. and G.M. Nomaguchi. Locus of adrenergic blocking action of dibenamine. *J. Pharmacol. Exp. Therap.* **93**:40, 1948.

107. Nystrom, R.A. Nervous control of the nictitating membrane. *Amer. J. Physiol.* **202**:849, 1962.

108. Ohlin, P. and B.C.R. Stromblad. Observations on the isolated vas deferens. *Brit. J. Pharmacol.* **20**:299, 1963.

109. Ottis, K., J.E. Davis and H.D. Green. Effects of adrenergic and cholinergic drugs on spleenic inflow and outflow before and during adrenergic blockade. *Amer. J. Physiol.* **189**:599, 1957.

110. Rand, M.J. and B.C. Whaler. Impairment of sympathetic transmission by botulinum toxin. *Nature.* **206**:588, 1965.

111. Rice, A.J. and J.P. Long. An unusual vasoconstriction induced by acetylcholine. *J. Pharmacol. Exp. Therap.* **151**:423, 1966.

112. Riley, M.W. and E.F. Maanen. Responses of guineapig vas deferens to autonomic drugs. *Fed. Proc.* **22**:170, 1962.

113. Rogers, D.K. and F.E. Leaders. Search for a cholinergic component in the sympathetic nerves to the perfused rabbit ear. *Arch. int. Pharmacodyn.* **163**:20, 1966.

114. Secker, J. The chemical agent in the sympathetic control of retraction of the nictitating membrane of the cat. *J. Physiol.* **89**:296, 1937.

115. Sherif, M.A.F. The chemical transmitter to the sympathetic nerve to the uterus. *J. Physiol.* **85**:298, 1935.

116. Shute, C.C.D. and P.K. Lewis. Cholinesterase-containing system of the brain of the rat. *Nature.* **199**:1160, 1963.

117. Singh, G.S. Action of choline on the rat blood pressure after various blocking agents. Paper presented at the XII annual conference of the Association of Physiologists and Pharmacologists of India, held at Patna, 1966.

118. Sinha, J.N., K.N. Dhawan, O. Chandra and G.P. Gupta. Role of acetylcholine in central vasomotor regulation. *Canad. J. Physiol. Pharmacol.* **45**:503, 1967.

119. Sjostrand, N.O. Effect of some smooth muscle stimulants on the motor response of the vas deferens to hypogastric nerve stimulation. *Nature.* **192**:1190, 1961.

120. Sjostrand, N.O. Inhibition by ganglion blocking agents of the motor response of the isolated guineapig vas deferens to hypogastric nerve stimulation. *Acta, Physiol. Scand.* **54**:306, 1962.

121. Srimal, R.C., B.P. Jaju, J.N. Sinha, K.S. Dixit and K.P. Bhargava. An analysis of the central vasomotor effects of choline. *European. J. Pharmacol.* **5**:239, 1969.

122. Thies, R.E. and V.B. Brooks. Post-synaptic neuromuscular block produced by hemicholinium. *Fed. Proc.* **20**:569, 1961.

123. Thompson, J.W. Studies on the responses of the isolated nictitating membrane of the cat. *J. Physiol.* **141**:46, 1958.

124. Trendelenburg, U. The action of histamine and 5-hydroxytryptamine on isolated mammalian heart. *J. Pharmacol. Exp. Therap.* **130**:450, 1960.

125. Trendelenburg, U. Modification of the effect of tyramine by various agents and procedures. *J. Pharmacol. Exp. Therap.* **134**:8, 1961.

126. Vincenzi, F.F. and T.C. West. Effect of hemicholinium on the release of autonomic mediators in the sinoatrial node. *Brit. J. Pharmacol.* **24**:773, 1965.

127. Vogt, M. Hypogastric nerve of dog. *Nature.* **193**:804, 1963.

128. West, T.C. Specialized tissues of the heart. Ed. A. Paes De Carvollio. p. 81, New York, Elsevier, 1961.

129. Wilson, W. and J.P. Long. The effect of hemicholinium (HC-3) at various peripheral cholinergic transmitting sites. *Arch. int. Pharmacodyn.* **120**:343, 1959.