MODIFICATION OF ADRENERGIC BLOCK BY NORADRENALINE LOW TEMPERATURE AND SODIUM DEFICIENCY

M.R. MANCHANDIA AND O.D. GULATI

Department of Pharmacology, Medical College, Baroda

Summary: Low temperature (0° - 10°C) could prevent but not reverse the adrenergic neurone blocking action of bretylium, xylocholine, bethanidine, debrisoquin, guanoxan and pyrilamine in periarterially stimulated rabbit isolated ileum preparation. Noradrenaline could prevent but not reverse the neurone blocking action of xylocholine, bethanidine and pyrilamine but failed to prevent or reverse that of bretylium, guanoxan and debrisoquin. Sodium-free solution could prevent but not reverse the neurone blocking action of xylocholine and pyrilamine. Sodium-free medium prevented as well as reversed the neurone blocking action of bethanidine, while it failed to prevent or reverse that of bretylium, guanoxan and debrisoquin. It is concluded that all agents are actively taken up by the adrenergic neurone and that xylocholine, bethanidine and pyrilamine share the uptake mechanism for noradrenaline.

Key words : adrenergic neurone blockers sodium-lack low temperature noradrenaline prevention

INTRODUCTION

The uptake of noradrenaline (NA) across the adrenergic neuronal membrane is energy-dependant (9, 12, 23) and sodium-dependant (2). This NA uptake mechanism is employed by guanethidine for its transport across the adrenergic neuronal membrane (7, 15) and human platelets (4) which can serve as convenient model for the neurone (20). Support for this common transport for NA and guanethidine is derived from observations on the ability of NA to block the uptake of guanethidine and the failure of uptake of guanethidine in sodium-deficient medium and under conditions of reduced energy supply as at low temperature (4).

Gulati and Jaykar (13) showed that in the Finkleman preparation (10), the neurone blocking action of guanethidine was prevented by NA, sodium-deprivation and at low temperature and concluded that guanethidine shares the uptake mechanism of NA. Since the neurone blocking action of a neurone blocker succeeds its uptake by the neurone, the prevention of block by various procedures affecting the uptake of NA should provide clue about the uptake of the neurone blocker. Thus, the present communication is concerned with the investigation of the influence of NA, low temperature and sodium-deprivation in modifying the neurone blocking action in Finkleman preparation (10) of bretylium, xylocholine, bethanidine, debrisoquine and guanoxan. The antihistaminic, pyrilamine was also included in the study since Verma and Gulati (22) showed that pyrilamine prevents the adrenergic neurone blocking action of guanethidine and agents which prevent the neurone blocking action of guanethidine are known to exhibit a neurone blocking action of their own (8).

MATERIALS AND METHODS

Segments of terminal ileum (2-3 cm long) from adult rabbits of either sex weighing 1.5-2 kg, were prepared with their sympathetic nerves intact by the method of Finkleman (10) and set up in a 33 *ml* organ bath containing McEwen's solution at 35.5±1°C. The details of the stimulation parameters and the method of recording were the same as those described by Gulati and Jaykar (13). Two preparations from the same animals were set up at the same time. After control responses to different frequencies had been recorded, one preparation (test) was subjected for a specified period of time to any one of the procedures described below. This was followed by the administration to both preparations of bethanidine (1.7×10^{-5}M) or xylocholine (5×10^{-5}M)or pyrilamine (2.5×10^{-5}M) or bretylium (2.5×10^{-5}M) or guanoxan (1.6×10^{-5}M) or debrisoquin (2.8×10^{-5}M). The period of exposure for bethanidine was 10 min and that for other blockers was 30 min. The preparations were then washed 6-8 times and responses of both the preparations to different frequencies were obtained once more. The various procedures employed were: (i) addition to the bath of NA (6×10^{-4}M) immediately before the neurone blocker; (ii) exposure of the preparations for 30 min to McEwen's solution at 0°C to 10°C and (iii) exposure of the preparations for 30 min to sodium-free McEwen's solution, the osmotic pressure and pH of which were maintained with sucrose (2.7×10^{-1}M) and $KHCO_3$ (2.5×10^{-3}M) respectively.

After the blocking effects of the neurone blockers had been studied in control preparations, they were subjected to any one of the procedures described above, the object being to determine the ability of these procedures to reverse the neurone blockade. The period of exposure was 20 min for NA and 40-60 min for other procedures. The preparations were then subjected to 6-8 washouts with McEwen's solution at 35.5°C followed by redetermination of the frequency-response curves.

Drugs: Bethanidine sulphate, bretylium tosylate, debrisoquin sulphate, guanoxan sulphate, (—) - noradrenaline (NA), pyrilamine maleate and xylocholine hydrochloride.

The solution of NA was prepared in distilled water which contained 0.05% sodium metabisulphite.

RESULTS

Adrenergic neurone blocking action of different blockers: Xylocholine and bretylium increased the basal tone; complete restoration occured after 6-8 washes; other blockers did not affect the tone or the pendular movements.

Bethanidine, bretylium, debrisoquin and guanoxan produced substantial to complete block of relaxation responses to periarterial sympathetic nerve stimulation at frequencies ranging from 1 to 20 Hz. Pyrilamine produced complete block in 11 out of 24 preparations, no block being obtained in the remainder of the preparations. Further, when a piece of ileum from one rabbit was blocked by pyrilamine, other pieces from the same ileum were also blocked. When

pyrilamine failed to block a piece of ileum from an animal the other pieces from the same animal were also not blocked. Xylocholine produced complete block in 5 out of 10 preparations; in 5 other preparations, the block was complete at lower frequencies (2 and 5 Hz) and partial (50-75 percent) at higher frequencies (10 and 20 Hz). The block lasted for more than 3 hrs in all cases. During the block, the preparations were as responsive to NA ($6x10^{-8}$M to 6×10^{-7} M) as during the pre-block period.

Effect of low temperature on the adrenergic neurone blocking action of different blockers: At low temperature the tissue was relaxed and the pendular movements were totally inhibited. Responses to nerve stimulation were absent during exposure to low temperature. Exposure of the tissue to medium at 35.5°C resulted in restoration of tone, pendular movements and responsiveness to nerve stimulation.

In general, hypothermia prevented the neurone blocking action of all the drugs and the prevention was greater with increasing hypothermia. The neurone blocking action of xylocholine was completely prevented at 5°C at all frequencies (n=3). A marked reduction in the neurone blocking action of bethanidine at 5°C and 10°C (n=3 each), of pyrilamine at 10°C (n=4), of bretylium at 5°C and 10°C (n=3 each) and of debrisoquin at 0°C and 5°C (n=3 each) was observed at higher frequencies but not at lower frequencies (Fig.1.) The neurone blocking action of guanoxan was partially reduced at 5°C and markedly reduced at 0°C (n=3 each) at higher fequencies but not at lower frequencies (Fig. 1.).

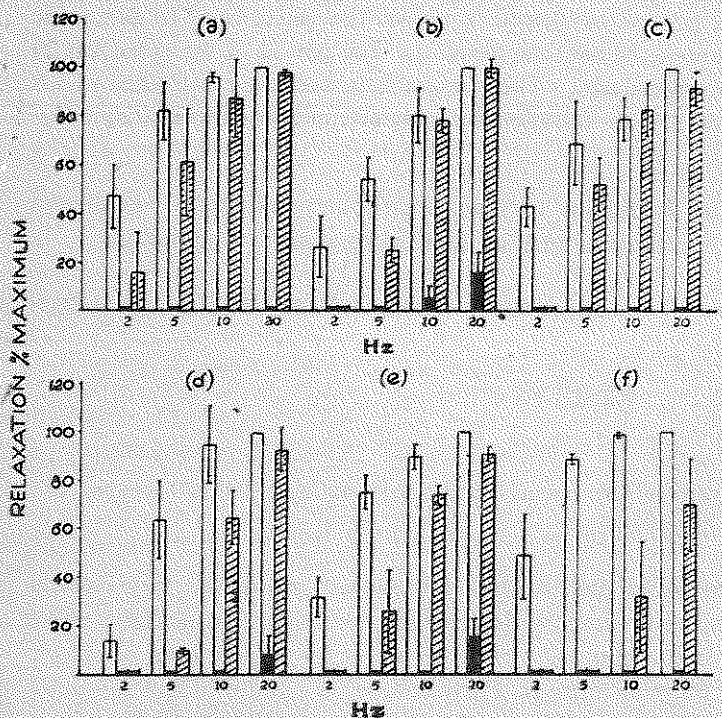

Fig. 1: Modification by low temperature of the effects of xylocholine ($5x10^{-5}$M; a), bethanidine ($1.7x10^{-5}$ M; b), pyrilamine ($2.5x10^{-5}$M; c), bretylium ($2.5x10^{-5}$M; d), debrisoquin ($2.8x10^{-5}$M;e) and guanoxan ($1.6x10^{-5}$M; f) on inhibitory responses to periarterial nerve stimulation (5 msec for 45 sec every 4 min at frequencies indicated below each panel in Hz) of paired (control and test) Finkleman preparations. All responses were recorded after equilibrating the preparations wih McEwen's solution at 35.5°C. Open columns indicate control reponses and closed columns indicate responses elicited after exposure of control preparations to blockers. Hatched columns indicate the prevention of neurone block in test preparations exposed to solution at 0°C in panel (f), 5°C in panels (a) and (e), and 10°C in panels (b), (c) and (d). Vertical lines indicate S.E.M.

The neurone blocking action of none of the agents was reversed by low temperature (0°C—10°C).

Effect of NA on the adrenergic neurone blocking action of different blockers: When NA was added to the bath, followed immediately by the addition of the adrenergic neurone blocking agent, the gut relaxed and the pendular movements were abolished. Complete restoration occured after 6-10 washouts.

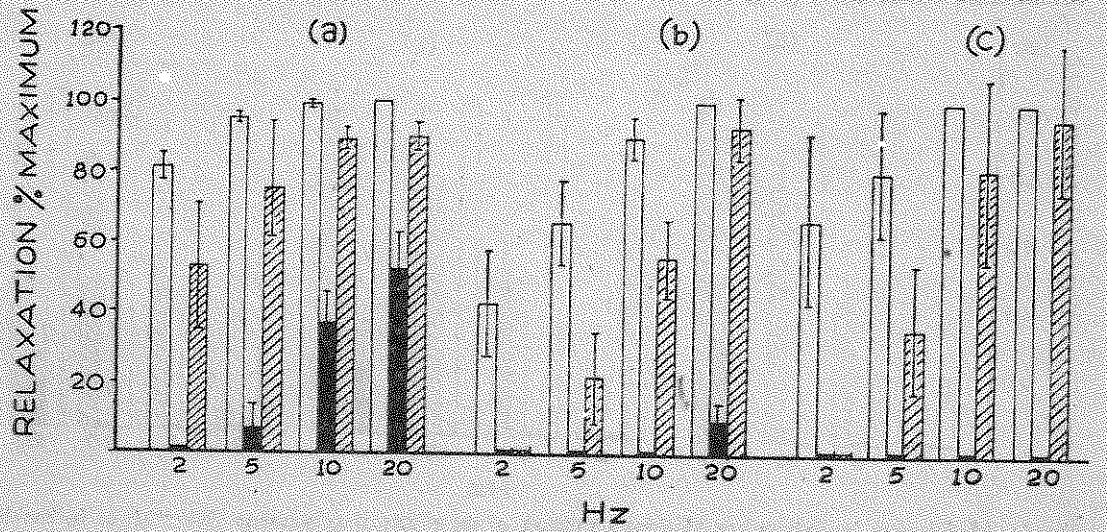

Fig. 2: Modification by noradrenaline (NA; $6x10^{-4}$M) of the effects of xylocholine ($5x10^{-5}$M; a) bethanidine ($1.7x10^{-5}$M; b) and pyrilamine ($2.5x10^{-5}$M; c) on inhibitory responses to periarterial nerve stimulation (5 msec for 45 sec every 4 min at frequencies indicated below each panel in Hz) of paired (control and test) Finkleman preparations bathed in McEwen's solution at 35.5°C. Open columns indicate control responses and closed columns indicate responses elicited after exposure of control preparations to blockers. Hatched columns indicate the prevention of neurone block in test preparations by NA. Vertical lines indicate S.E.M.

NA markedly prevented the neurone blocking action of xylocholine (n=4), bethanidine (n=4) and pyrilamine (n=3) (Fig. 2). The reduction was more marked at higher frequencies than at lower frequencies. The neurone blocking action of bretylium (n=3), guanoxan (n=3) and debrisoquin (n=6) was not affected.

NA failed to reverse the neurone blocking action of any of the blockers.

Effect of sodium-free solution on the adrenergic neurone blocking action of different blockers:

Exposure of ileum to the sodium-free McEwen's solution for 30 min produced slight relaxation and the inhibitory responses to nerve stimulation could not be elicited. Complete restoration followd 6-8 washouts with normal McEwen's solution.

Following prior exposure to sodium-free medium there was marked reduction in the

neurone blocking action of xylocholine (n=3), bethanidine (n=3) and pyrilamine (n=4) (Fig. 3). The neurone blocking action of guanoxan, debrisoquin and bretylium (n=4 each) was not affected.

There was no reversal of the neurone blocking action of xylocholine, bretylium, guanoxan and debriscquin by sodium-free solution, However, the neurone blocking action of bethanidine was reversed (Fig. 3).

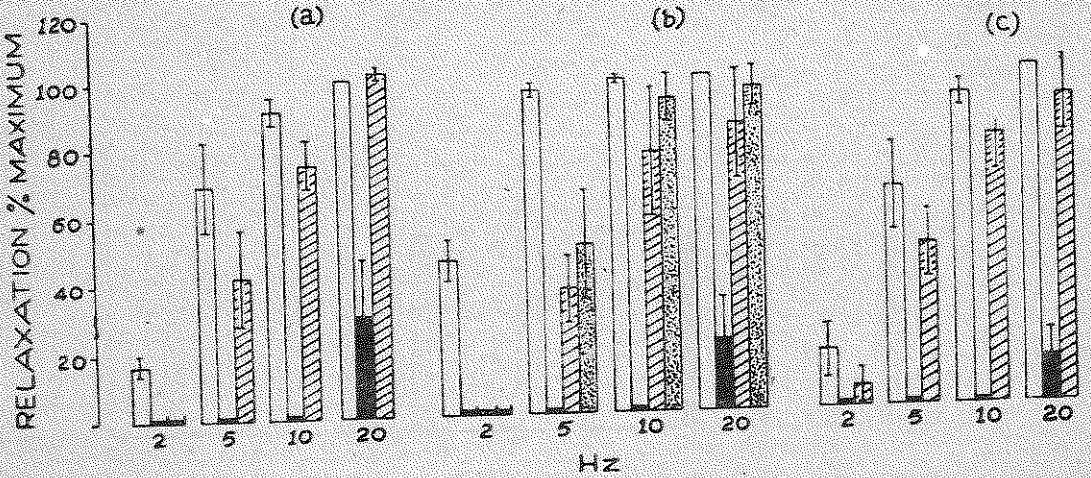

Fig. 3: Modification by sodium-free McEwen's solution of the effects of xylocholine ($5x10^{-5}$M; a), bethanidine ($1.7x10^{-5}$M; b) and pyrilamine ($2.5x10^{-5}$M; c) on inhibitory responses to periartial nerve stimulation (5 msec for 45 sec every 4 min at various frequencies indicated below each panel in Hz) of paired (control and test) Finkleman preparations. All responses were recorded after equilibrating the preparations with McEwen's solution at 35.5°C. Open columns indicate control responses and closed columns indicate responses elicited after exposure of control preparations to blockers. Hatched columns indicate the prevention of neurone block in test preparations by sodium-free solution. Stippled columns in (b) indicate the reversing action elicited by exposure of control preparations to sodium-free solution for 40 min after inducing neurone block with bethanidine. Vertical lines indicate S.E.M.

DISCUSSION

The uptake of NA by the adrenergic neuronal membrane has been shown to be energy-dependent (9, 12, 23). In the present study the adrenergic neurone blocking action of all the blockers was substantially prevented at low temperature. This observation would suggest that the blockers were predominently taken up actively by the adrenergic neurone. Since the prevention was not complete, it is possible that some passive diffusion of the blockers into the neurone may also be involved. Support for this suggestion is derived from the results of Reitz *et al.*, (18) who observed a passive diffusion of NA into mouse cerebral cortex.

NA and sodium-deprivation prevented the adrenergic neurone blocking action of xylocholine, bethanidine and pyrilamine. It is possible that the prevention by NA could

atleast in part be due to its metabolites. Experiments with the inhibitors of the enzymes, MAO and COMT would serve to clarify the role if any of the metabolites of NA. Both NA and sodium-deprivation failed to prevent the adrenergic neurone blocking action of bretylium, guanoxan and debrisoquin. Since sodium ion is an absolute requirement for the uptake of NA in the adrenergic nerve endings (2), our results would suggest that xylocholine, bethanidine and pyrilamine share the uptake mechanism for NA. Toda (21) similarly failed to observe a preventing action of sodium-deficiency on the adrenergic neurone blocking action of bretylium in rabbit atria and rabbit ascending aorta and O'Brien and Boullin (16) observed only 31.2% inhibition of the uptake of debrisoquin by human platelets with ouabain which inhibits sodium potasium-dependent ATP-ase (11) and, therefore, reduces extracellular sodium concentration. It is conceivable, therefore, that bretylium, guanoxan and debrisoquin are transported across the adrenergic neurone by some mehanism distinct from the one concerned with the transport of NA, a suggestion already made for bretylium and debrisoquin (6, 17, 21).

NA or low temperature failed to reverse the neurone blocking action of any of the blockers. Exposure to sodium-free solution reversed only the adrenergic neurone blocking action of bethanidine. This was surprising since sodium deficiency does not reverse the adrenergic neurone blocking action of guanethidine (13) which is closely allied chemically to bethanidine and both drugs deplete NA stores (5). Certain differences between the two drugs have, however, recently been suggested. The existence of two pools of guanethidine has been demonstrated in the rat heart by Boullin (3). Shand *et al.*, (19) suggested that guanethidine and bethanidine are taken up into two pools within the adrenergic neurone and intravesicular and an extravesicular pool. The extravesicular pool contains the drug in more ready equilibrium with the perfusate and its efflux is relatively rapid. The disappearace from the intravesicular pool is slow, despite deminished concentrations of these blocking drugs in the perfusate. It was further suggested that with the concentrations of bethanidine required for complete blockade there was lesser uptake of bethanidine into the intravesicular pool. Sodium-deficiency causes loss of NA from adrenergic nerve endings (1, 2). If it could be shown that the extravesicular pool is more sensitive to sodium-deficiency than the intravesicular pool the reversal of the neurone blocking action of bethanidine by sodium deficiency would become easily understandable.

ACKNOWLEDGEMENTS

It is pleasure to express our thanks to Ciba of India (Bombay), for bethanidine sulphate; Mr. A.F. Green of The Wellcome Research Laboratories (Beckenham), for bretylium tosylate; to Roche Products Limited (Bombay), for debrisoquin sulphate; to Pfizer Limited (Bombay), for guanoxan sulphate; to Mr. A.V. Mody of Unichem Laboratories (Bombay), for noradrenaline; to May & Baker Limited (Bombay), for pyrilamine maleate and to Mr. Gordon Wilfon of Smith Kline and French Laboratories (Philadelphia), for xylocholine hydrochloride. This work was supported by a research grant from the Council of Scientific and Industrial Research, New Delhi.

REFERENCES

1. Bogdanski, D.F. and B.B. Brodie. Role of sodium and potassium ions in storage of norepinephrine by sympathetic nerve endings. *Life Sci.*, **5** : 1563-1569, 1966.
2. Bogdanski, D.F. and B.B. Brodie. The effect of inorganic ions on the storage and uptake of H^3—norepinephrine by rat heart slices. *J. Pharmac Exp. Ther.*, **165** : 181-189, 1969.
3. Boullin, D.J. Evidence for intracellular guanethidine stores in the rat heart revealed after removal of calcium ions. *Br. J. Pharmac.*, **32** : 145-155, 1968.
4. Boullin, D.J. and R.A. O'Brien. The accumulation of guanethidine by human blood platelets. *Br. J. Pharmac.*, **35** : 90-102, 1969.
5. Boura, A.L.A., F.C. Copp, A.F. Green, H.F. Hodson, G.K. Ruffella, M.F. Sim, E. Walton and E.M. Grivsky. Adrenergic neurone blocking agents related to choline 2,6-xylylether bromide (TMIO) bretylium and guanethidine. *Nature* (*Lond.*), **191** : 1312-1313, 1961.
6. Brodie, B.B., C.C. Chang and E. Costa. On the mechanism of action of guanethidine and bretylium. *Br. J. Pharmac.*, **25** : 171-178, 1965.
7. Chang, C.C., E. Costa and B.B. Brodie. Interaction of guanethidine with adrenergic neurone. *J. Pharmac. Exp. Ther.*, **147** : 303-312, 1965.
8. Day, M.D. and M.J. Rand. Evidence for a competitive antagonism of guanethidine by dexamphetamine. *Br. J. Pharmac.*, **20** : 17-28, 1963.
9. Dengler, H.J., I.A. Michaelson, H.E. Spiegel and E.O. Titus. The uptake of labelled norepinephrine by isolated brain and other tissues of the cat. *Int. J. Neuropharmac.*, **1** : 23-30, 1962.
10. Finkleman, B. , On the nature of inhibition in the intestine. *J. Physiol.* (*Lond.*), **70** : 145-157, 1930.
11. Garrahan, P.J. and I.M. Glynn. The stoicheiometry of sodium pump. *J. Physiol.* (*Lond.*), **192** : 217-235, 1967.
12. Green, R.O. and J.W. Miller. Evidence for the active transport of epinephrine and norepinephrine by the uterus of the rat. *J. Pharmac. Exp. Ther.*, **152** : 42-50, 1966.
13. Gulati, O.D. and S. Jaykar. Factors affecting the action of guanethidine on adrenergic neruones. *Br. J. Pharmac.*, **42** : 352-363, 1971.
14. McEwen, L.M. The effect on the isolated rabbit heart of vagal stimulation and its modification by cocaine, hexamethonium and ouabain. *J. Physiol.* (*Lond.*), **131** : 678-689, 1956.
15. Obianwu, H.O., R Stitzel and P. Lundborg. Subcellular distribution of H^3 amphetamine and H^3 guanethidine and their int raction with adrenergic neurone. *J. Pharm. Pharmac.*, **20** : 585-594, 1968.
16. O'Brien, R.A. and D.J. Boullin. Accumulation, storage and release of adrenergic neurone blocking agents and related drugs by human platelets. *Biochem. Pharmac.*, **21** : 1817-1827, 1972.
17. Prasad, C.M., D.S. Shah and O.D. Gulati, Some factors affecting the neurone blocking action of xylocholine, bretylium and debrisoquin. *Jap. J. Pharmac.*, (in press).
18. Reitz, R.H., S.E. Bocknik and S.A. Kulkarni, A comparison of Mgpemoline and (+)—amphetamine effects upon avoidance behaviour and amine pump. *J. Pharm. Pharmac.*, **24** : 830-832, 1972.
19. Shand, D.G., D.H. Morgan and J.A. Oates. The release of guanethidine and bethanidine by splenic nerve stimulation : a quantitative evaluation showing dissociation from adrenergic blockade. *J. Pharmac. Exp. Ther.*, **184** : 73-80, 1973.
20. Solomon, H.M., C. Ashley, N Spirt and W.B. Abrams. The influence of debrisoquin on the accumulation and metabolism of biogenic amines by the human platelet, *in vivo* and *in vitro*. *Clin. Pharmac. Ther.*, **10** : 229-238, 1969.
21. Toda, N. Interactions of bretylium and drugs that inhibit the neuronal membrane transport of norepinephrine in isolated rabbit atria and aorta. *J. Pharmac. Exp. Ther.*, **181** : 318-321, 1972.
22. Verma, B.S. and O.D. Gulati. Mepyramine and adrenergic neurone. XIII *Congressus Internationalis Dermatologir*-Berlin, Heidelberg, New York, Springer Verlag, 1968, p. 1053-1056.
23. Wakade, A.R. and R.F. Furchgott. Metabolic requirements for the uptake and storage of norepinephrine by the isolated left atrium of guinea pig. *J. Pharmac. Exp. Ther.*, **163** : 123-135, 1968.