

the resulting response to ICV injection of NE may depend on the balance between the effect of receptors responsible for the pressor and depressor responses in the central nervous system (CNS) controlling blood pressure.

In anaesthetized rabbits, yohimbine an alpha-2 blocker as well as metoprolol, a beta-1 blocker abolished the hypotensive response of ICV injection of NE (9). From this study, it is difficult to resolve the contribution of individual receptor type as pretreatment with yohimbine as well as with metoprolol produced 100% blockade of NE response. Since the role of specific central adrenoceptors (alpha-1, alpha-2, beta-1, beta-2) in the control of blood pressure is also not clearly defined by the previous studies, the present investigation was undertaken to examine:

- (i) The effect of ICV injection of NE on BP and HR in conscious rabbits to eliminate the influence of anaesthesia on the responses.
- (ii) Influence of selective blockers alpha-1, Prazosin; alpha-2, Yohimbine; beta-1, Metoprolol; beta-2, Butoxamine on the response of ICV injection of NE in these rabbits.

METHODS

The present study was conducted in 19 conscious rabbits (*Oryctolagus cuniculus*) of either sex weighing 1.5 to 2.5 kg. The animals were anaesthetized using urethane (2 g/kg body wt., ip) for implanting chemitrode into the lateral ventricle (10). One to two week after this surgical procedure, one of the central ear artery was cannulated using polyethylene catheter (1 mm int. diameter) under local anaesthesia (1% lignocaine). The other end of arterial cannula was connected to a pressure transducer (Statham P23DC) for recording BP on a Polygraph (Beckman R-611 Dynograph, USA) with the help of voltage/pulse/pressure coupler (Beckman Model 1 No. 215606). Heparinized saline solution (500 IU/ml normal saline) was administered i.v. in small amounts and was also used for rinsing the pressure transducer arterial catheter

assembly to prevent formation of any blood clots. A 16 mm long 25 gauge needle was inserted through the guide tube into the lateral ventricle to check the free flow of CSF through the needle in the lateral ventricle. Dead space of the needle was measured before its implantation and this was added to the actual volume of ICV injection of saline, NE and all selective blockers used in this study. BP of the rabbit was recorded at a recorder paper speed of 2.5 mm/sec. The pressure recording system was calibrated with a mercury manometer. Using a microlitre tuberculin syringe, 20 µl normal saline was injected into the ventricle and BP was recorded. This record acted as a control for subsequent ICV injections of drugs. Volume of the drugs injected was kept same (20 µl) throughout the experiment.

The dose-responses of centrally administered NE on BP and HR were studied by ICV administration of NE in incremental doses by same amount every 5 min to a maximum of 40 µg. NE response was found to be reproducible in each animal over time provided the subsequent injections were given after the recovery from the earlier injection of NE. In order to investigate the role of specific adrenoceptors, selective adrenergic blockers (prazosin, yohimbine, metoprolol, butoxamine) were administered ICV in the dose of 20 µg and followed by 20 µg NE immediately and after intervals of 5, 10 and 15 min thereafter in the same animal.

In order to test the possibility of any leakage of ICV injected drugs into systemic circulation, Evans blue dye (20 µl) was administered ICV. The arterial blood samples were collected immediately after injection of Evans blue dye then at 30 sec, 1 min and 5 min. Spectrophotometric analysis of arterial blood samples did not show any leakage or ICV administered NE from the brain into the systemic circulation.

Drugs :

NE (Unichem Laboratory) and adrenoceptor antagonists used were prazosin (Pfizer Ltd.), yohimbine (Sigma), metoprolol (Astra-IDL Ltd.) and butoxamine (Wellcome Research

Laboratories). All the drugs were dissolved in normal saline before the experiment.

Statistical analysis of the data :

The data was subjected to analysis of variance in a randomized block animals arrangement after ascertaining the homogeneity of variance and normality. The data from each series of experiments were analysed separately. On evidence of significant effects individual and other appropriate comparisons were done through linear contrast (11). The variables have been expressed throughout as mean values and the variation between the animals of the same group is indicated by standard deviation (\pm SD).

RESULTS

(a) Effect of ICV administration of normal saline:

ICV administration of 20 μ l of normal saline in conscious rabbits had no effect on BP and HR (Fig. 1). Therefore, 20 μ l of normal saline was used as control in each experiment. The volume of NE and all the adrenoceptor selective blockers was kept same (20 μ l) throughout the study.

(b) Effect of ICV injection of NE on BP and HR:

The resting average systolic blood pressure was 110 ± 3.4 mmHg, diastolic blood pressure was 71 ± 4.8 mmHg and mean blood pressure was 84 ± 3.8 mmHg and mean resting HR was 194 ± 7 beats/min (Fig. 1). A significant ($P<0.05$) fall in both systolic and diastolic BP was observed in ICV injection of NE (Fig. 1). Both systolic and diastolic BP showed almost equal fall of about 30 mmHg from their corresponding resting values. The BP started falling 2.0 \pm 0.9 sec after

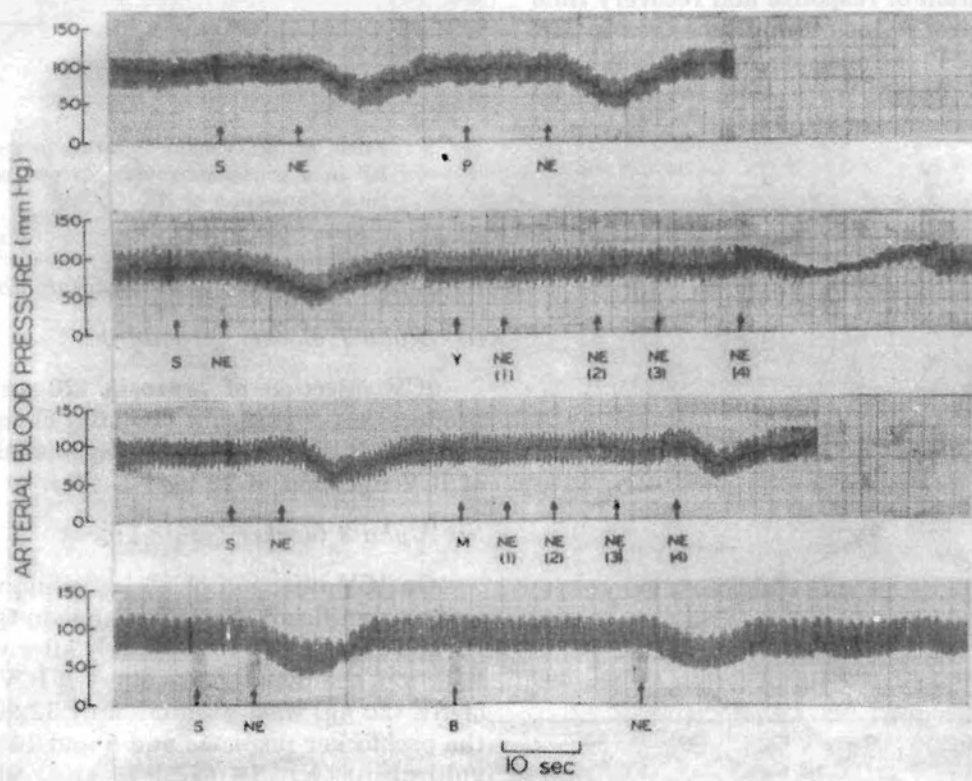


Fig. 1 : Effect of ICV administration of Saline (S), Norepinephrine (NE), Prazosin (P); Yohimbine (Y), Metoprolol (M) and Butoxamine (B) on Systolic, Diastolic and Mean arterial blood pressure (original representative record).

injection of NE and remained at lower level for another 8.0 ± 0.19 sec : thereafter, it gradually returned to control value after 10.0 ± 0.24 sec. Average systolic BP after ICV injection of NE was 77 ± 4.4 mmHg, diastolic BP was 41 ± 3.9 mmHg and mean BP was 53 ± 4.1 mmHg. HR did not show any significant ($P > 0.05$) difference from its resting mean value of 194 ± 3 beats/min. After ICV injection of NE, mean HR was 198 ± 3 beats/min (Fig. 1).

(c) *Dose-response of NE :*

ICV administered NE in incremental doses (10, 20, 30 and 40 μ g), produced depressor response in a dose-dependent manner. Resting mean BP was 84 ± 3.8 mmHg and with incremental doses of NE, it dropped to 61 ± 3.5 , 53 ± 4.1 , 45 ± 4.0 and 39 ± 3.5 mmHg respectively (Fig. 2). However, no changes were observed in latency, duration of response and recovery time of BP to control values. Small changes in HR following ICV administration of NE in incremental doses were not found to be statistically significant ($P > 0.05$) (Fig. 2).

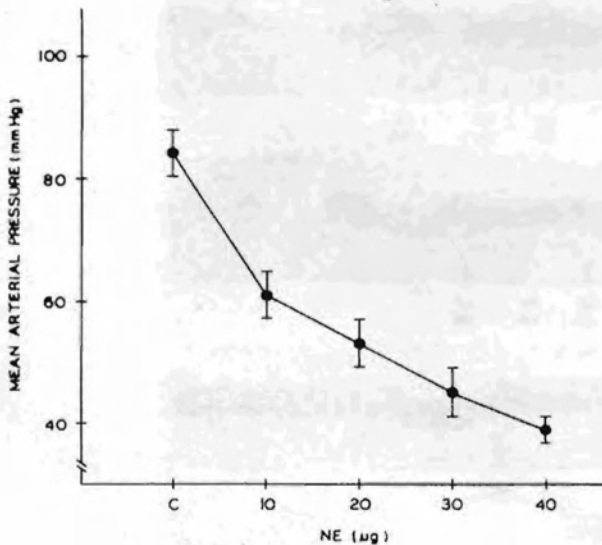


Fig. 2 : Effect of ICV administration of different doses of NE on mean arterial pressure. Values are mean \pm SEM.

(d) *Effect of intravenous administration of NE on BP and HR :*

Intravenous administration of NE (20 μ g) produced a marked increase in BP (both systolic and diastolic) and a significant ($P < 0.05$) fall in HR. Mean BP increased to 140 ± 4.1 mmHg from its resting mean value of 82 ± 4.6 mmHg and a significant ($P < 0.05$) fall in HR from 194 ± 7 to 37 ± 9 beats/min occurred. The effect occurred instantaneously and persisted for 3.0 ± 0.14 sec then gradually returned to the control level after 4.2 ± 0.17 sec (Fig. 3).

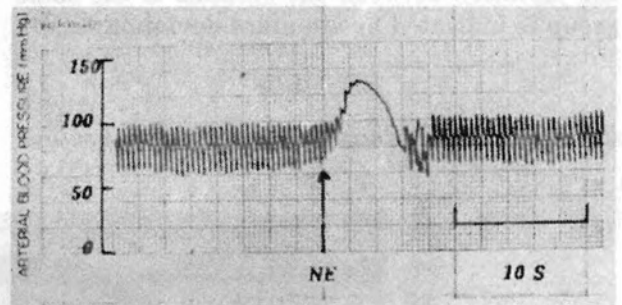


Fig. 3 : Effect of intravenous injection of 20 μ g NE on BP in a conscious rabbit. Arrow indicates the time of injection of NE.

(e) *Effect of selective adrenoceptor blocker :*

(i) *Alpha-1 blocker (prazosin) :*

ICV injection of prazosin (20 μ g) did not produce any significant ($P > 0.05$) change in BP and HR. It did not alter the hypotensive action of ICV injection of 20 μ g NE (Fig. 4).

(ii) *Alpha-2 blocker (yohimbine) :*

On ICV injection of 20 μ g yohimbine there was no significant ($P > 0.05$) change in the resting BP and HR (Fig. 4). Immediately after yohimbine injection, the hypotensive effect of ICV injection of NE (20 μ g) was attenuated by $12.90 \pm 0.3\%$ of the preblocker response and 5 and 10 min after yohimbine by $38.7 \pm 0.9\%$ and $51.6 \pm 0.2\%$ respectively. Fifteen min after yohimbine, NE (ICV) response was attenuated by $(74.2 \pm 0.7\%)$ which is shown in Fig 2 and 4. However, no

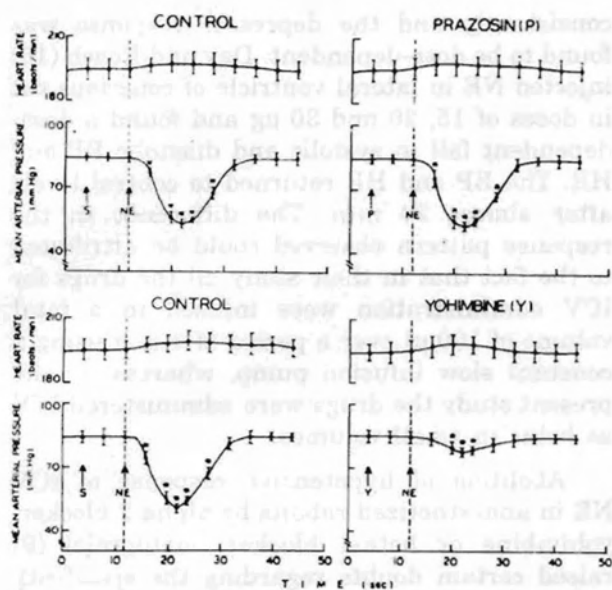


Fig. 4 : Effect of ICV administration of NE (20 µg) before and 15 min after ICV administration (20 µg) of prazosin and yohimbine on Mean Blood Pressure and Heart Rate. Values are mean±SEM.

changes were observed in HR due to ICV injection of yohimbine (20 µg) or by injection of 20 µg of NE immediately or 5, 10 and 15 min after yohimbine.

(iii) *Beta-1 blocker (metoprolol) :*

There was no significant ($P>0.05$) change in BP on ICV injection of metoprolol (20 µg) (Fig. 6). The NE (ICV) action on BP was blocked by $9.4\pm 0.04\%$ immediately after metoprolol and by 12.5 ± 0.3 and $18.8\pm 0.8\%$, 5 and 10 min after metoprolol respectively. Blockade of NE response 15 min after metoprolol was $25\pm 0.5\%$ (Fig. 5). HR did not show any change either due to ICV injection of metoprolol or by NE injection immediately and at intervals of 5, 10 and 15 min after metoprolol.

(iv) *Beta-2 blocker (butoxamine) :*

ICV injection of beta-2 blocker butoxamine did not affect BP and HR and it did not produce any significant ($P>0.05$) change in BP response to NE (ICV) (Fig. 6).

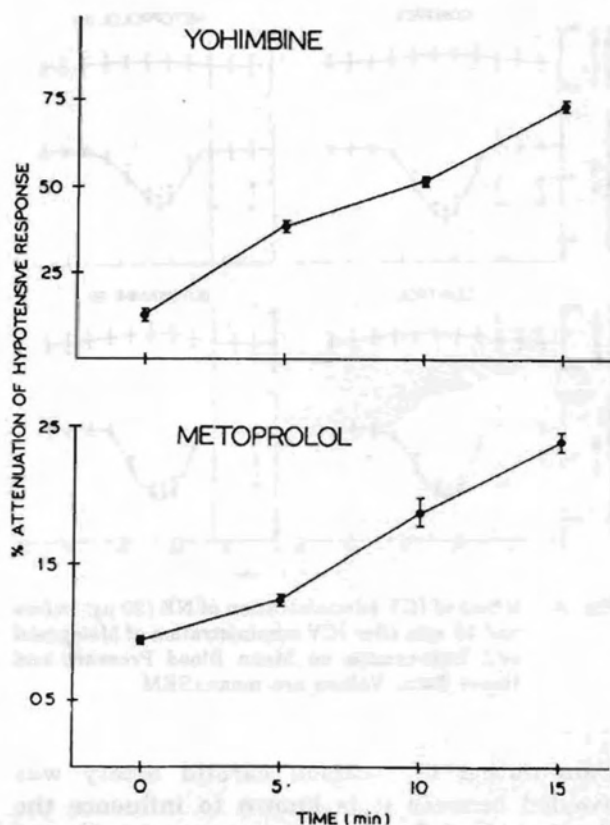


Fig. 5 : Percent attenuation in hypotensive response of 20 µg ICV NE in a conscious rabbit with time after treatment with ICV 20 µg yohimbine (upper trace) and 20 µg metoprolol (lower trace). The values at 0 time represent attenuation of ICV NE response immediately after injection of blockers : Yohimbine/metoprolol. Values are mean±SEM.

DISCUSSION

The present study elucidated the role of central adrenoceptors (alpha-1, alpha-2, beta-1 and beta-2) in the hypotensive response of ICV injection of NE in awake rabbits. As in the periphery four types of adrenergic receptors have been described in the CNS i.e. alpha-1, alpha-2, beta-1 and beta-2 (12).

In the present study the central ear artery was preferred over common carotid artery or femoral artery for recording BP in conscious animals as it required much simpler surgical procedure, with minimal surgical trauma.

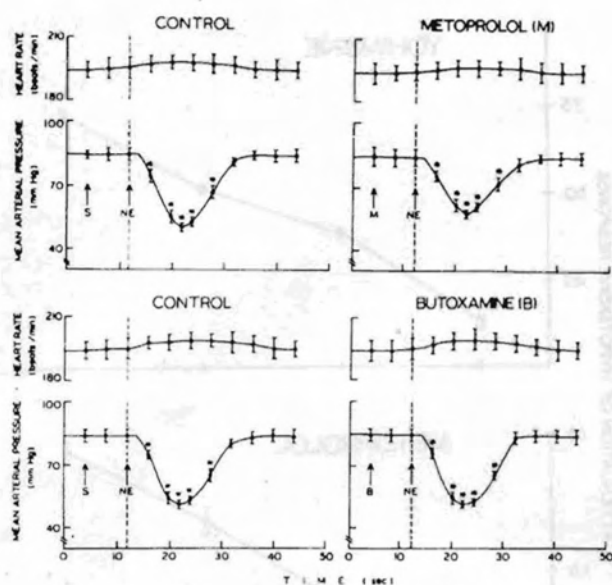


Fig. 6 : Effect of ICV administration of NE (20 μ g) before and 15 min after ICV administration of Metoprolol and Butoxamine on Mean Blood Pressure and Heart Rate. Values are mean \pm SEM.

Cannulation of common carotid artery was avoided because it is known to influence the cerebral blood flow leading to cerebral ischemia, which may alter the response of ICV injection of NE and other adrenoceptor blockers. Absence of HR response to hypotensive response of ICV injected NE could not be attributed to depressed baroreflex response because intravenous NE produced a significant increase in BP and a corresponding fall in HR.

In the earlier studies, distinctly contradictory response to ICV injection of NE on BP and HR have been reported. Some of the workers have observed a hypotensive response to ICV injection of NE (5, 13), while others (1) have reported a hypertensive response to ICV injection of NE. The hypothesis of a depressor central receptor presents some discrepancies. First of all such receptors are not well accounted and secondly ICV injection of NE has been reported to produce either elevation or fall in BP. Several factors must be taken into account in the analysis of such discrepancies. In our animals, ICV NE decreased BP (both systolic and diastolic)

consistently and the depressor response was found to be dose-dependent. Day and Roach (15) injected NE in lateral ventricle of conscious cat in doses of 15, 20 and 30 μ g and found a dose-dependent fall in systolic and diastolic BP and HR. The BP and HR returned to control levels after almost 30 min. The difference in the response pattern observed could be attributed to the fact that in their study all the drugs for ICV administration were infused in a total volume of 100 μ l over a period of 4 min using a constant slow infusion pump, whereas, in the present study the drugs were administered ICV as bolus in small volumes.

Abolition of hypotensive response of ICV NE in anaesthetized rabbits by alpha-2 blocker: yohimbine or beta-1 blocker: metoprolol (9) raised certain doubts regarding the specificity of the central adrenergic receptor involved in the hypotensive response of NE (ICV). However, the results of present investigation clearly demonstrated that the hypotensive response of ICV injected NE in conscious rabbits is mediated mainly through central alpha-2 adrenergic receptors and partly through beta-1 receptors. One of the possible reasons for variable responsiveness of ICV NE could be the influence of anaesthetic on the magnitude and the pattern of response.

Administration of exogenous adrenaline, which is the main endogenous beta-2 adrenoceptor agonist, increases blood flow in several vascular areas. The influence of non-selective beta-1 blockade and cardioselective beta-1 blockade on these responses has also been reported (14), which provided some qualitative information about the beta-2 adrenergic component in the response. However, the present study revealed that the hypotensive response to ICV injection of NE in conscious rabbits is mediated mainly through alpha-2 central adrenergic receptors and partly due to beta-1 adrenergic receptors.

Correa et al (15) postulated that the pressor response to ICV injected NE in awake rats was not mediated by sympathetic nervous system and involved release of pituitary humoral factors,

most probably vasopressin, whereas, the depressor response observed in anaesthetized animals was not dependent on pituitary mediation. Those workers further proposed the involvement of histaminergic mechanisms in the CNS in the control of the pressor response to ICV administrated NE in rats.

In the present study, maximum block of the depressor response of ICV NE by a selective dose of yohimbine (alpha-2 blocker) or metoprolol (beta-1 blocker) was observed 15 min after ICV administration of yohimbine, or metoprolol. The results demonstrated greater sensitivity of alpha-2 receptors to ICV NE.

The way drug treatments affect the response to centrally administrated catecholamine also represent an important feature in analysis of the differences in net results. Phentolamine in cats, has been shown to counteract the depressor response of NE by blocking marked changes in baseline BP. Phentolamine also blocks effects of clonidine on central nervous system and again the same explanation can be made (16). Clonidine has been shown to possess more alpha-2 than alpha-1 stimulating activity and its CNS actions are better blocked by piperoxan or yohimbine which are more specific blockers of alpha-2 adrenergic-receptors (16). The hypotensive action of clonidine had been explained on the basis of agonist activity at alpha-2 receptor site. The presynaptic beta receptors had been proposed to exert a facilitatory effect on neuronal NE release, an effect attenuated by beta-adrenergic antagonists (17). The alpha-2 nature of CNS hypotensive mechanism was also proposed (18). Here the same explanation as in case of phentolamine does not apply because the alpha-2 blocking agents do not interfere with the baseline BP to the same extent. However, piperoxan or yohimbine are effective blockers of clonidine but not epinephrine induced hypotension in rat. The observations of Brokoswki and Finch (19) present an interesting discrepancy which is against the idea of a general mediation of CNS hypotensive response by alpha receptors among various species.

In the rat, there is evidence pointing to alpha and beta receptors mediation of catecholaming induced hypotensive response. In this species the hypotensive response of ICV epinephrine but not of clonidine is blocked by beta-antagonists propranolol and metoprolol (15-16). The suggestion of a beta-adrenoceptor mediation of the hypotensive response of epinephrine can be extended to other catecholamines because ICV pretreatment with propranolol also blocked, even reversed the hypotensive response of NE into a hypertensive one (16). The possible involvement of pathways other than adrenergic in the cardiovascular responses to ICV injection of catecholamines such as histaminergic pathways could possibly explain the mediation of the hypotensive response to clonidine and the hypertensive response to ICV injection in unanaesthetized rats.

The findings that alpha adrenoceptor blocking agents blocked the bradycardia produced by NE and phenylephrine and beta blocking agents blocked the tachycardia produced by isoprenaline (5), suggest that activation of central alpha receptors induced bradycardia and activation of central beta receptors caused tachycardia.

In conclusion results of the present study demonstrated a dose-dependent hypotensive response to ICV injection of NE and no significant change in HR in awake rabbits. The hypotensive response was found to be mediated mainly through alpha-2 central adrenergic receptors and partly through central beta-1 adrenergic receptors.

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