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CENTRALLY INDUCED HYPOTENSION AND BRADYCARDIA AFTER ADMINISTRATION OF α-METHYLNORADRENALINE INTO THE AREA OF THE NUCLEUS TRACTUS SOLITARII OF THE RAT

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1 In anaesthetized rats, bilateral injections of α-methylnoradrenaline, noradrenaline or adrenaline into the area of the nucleus tractus solitarii (NTS) of the brain stem caused dose-dependent decreases of systemic arterial blood pressure and heart rate. The effects of α-methylnoradrenaline were most pronounced and lasted longest.

2 The cardiovascular effects of α-methylnoradrenaline appeared to be restricted to the medio-caudal part of the NTS.

3 Prior administration of the α-adrenoceptor blocking agent, phentolamine, reversed the fall in blood pressure and heart rate induced by α-methylnoradrenaline into an increase.

4 Systemic administration of atropine combined with vagotomy potentiated the inhibitory effects of α-methylnoradrenaline on the cardiovascular system.

Introduction

Evidence is accumulating for a centrally mediated blood pressure lowering effect of α-methyldopa (Van Zwieten, 1975). The metabolite which mediates the central effect on blood pressure is thought to be α-methylnoradrenaline since inhibition of central dopa-decarboxylase or dopamine-β-hydroxylase activity prevents the central hypotensive action of α-methyldopa (Henning & Rubenson, 1971; Day, Roach & Whiting, 1973; Henning, 1975; Nijkamp, Ezer & De Jong, 1975). Experiments in cats in which α-methyldopa was infused into the vertebral artery indicated that the main site of action may be located in the medulla oblongata (Henning & Van Zwieten, 1968). Further, we have shown (Nijkamp & De Jong, 1975), that local injection of α-methylnoradrenaline into the nucleus tractus solitarii (NTS) of the medulla oblongata caused a reduction in blood pressure and heart rate. Pressor doses of the peptides angiotensin or vasopressin were without effect. Injections or infusions of α-methyldopa, α-methyldopamine or α-methylnoradrenaline into the cerebral ventricles of anaesthetized or conscious cats or rats caused hypotensive responses which were prevented by α-adrenoceptor antagonists (Heise & Kroneberg, 1972; Day & Roach, 1974; Finch, Hersom & Hicks, 1975). The present study describes the site of action of α-methylnoradrenaline in the NTS and compares this action with that of noradrenaline and adrenaline. The results obtained are described here in detail supplementing a brief preliminary report which has already been made (De Jong, Nijkamp & Bohus, 1975a).

Methods

The experiments were performed on male Wistar rats (outbred stock, Wi/Cpb, Zeist) weighing 200–240 grams. The animals were anaesthetized with urethane (1.25 g/kg) and placed in a stereotaxic apparatus (David Kopf) with the head flexed to 45°. The dorsal surface of the lower brain stem was then exposed by a limited occipital craniotomy. The obex (corresponding with the rostral part of the area postrema) was used as a stereotaxic zero. Bilateral injections into the NTS (0.5 mm lateral to the obex and at a depth of 1.0 mm) were carried out with a stainless steel needle (outer diameter 0.2 mm) connected via a polyethylene cannula with an Agla micrometer syringe and a Shardlow micrometer. Injections in other sites in the NTS and outside the NTS were also given at a depth of 1 mm into the brain stem. Drugs were dissolved in 0.9% w/v NaCl solution (saline) and administered bilaterally in a volume of 0.6 μl over a period of 30 seconds. Control rats received 0.6 μl of saline bilaterally.

Blood pressure was continuously recorded from a permanent indwelling iliac cannula with a Statham transducer (Model P23AC) connected to a Grass polygraph. The iliac cannula had been implanted

under ether anaesthesia at least 24 h before the experiment (Nijkamp *et al.*, 1975). Heart rate was computed from the blood pressure pulse wave by a cardiotachometer (Narco Bio systems, model BT1200). Bilateral vagotomy was carried out under ether anaesthesia at mid-cervical level 30 min before local injection of α-methylnoradrenaline. In experiments in which vagotomy was performed, atropine (5 mg/kg i.p.) was given at the same time. Drugs given intravenously were dissolved in 0.2 ml saline and administered into the right jugular vein.

After termination of the experiments, the brains were fixed in 5% formalin. Frozen sections of 100 μm were cut and stained with 0.1% thionine. The injection sites were controlled microscopically.

Results are expressed as means ± standard error of the mean (s.e. mean). Significance of the difference between values of control and treated groups was determined with Student's *t*-test.

The following drugs were used: (–)-adrenaline bitartrate (Boehringer); atropine sulphate (OPG); (±)-erythro-α-methylnoradrenaline hydrochloride (Cobrefin, Winthrop Labs.); (–)-noradrenaline bitartrate (Levarterenol, OPG); phentolamine methane sulphonate (Regitine, Ciba); urethane (OPG). Drug weights refer to the salts except for those of adrenaline, noradrenaline and α-methylnoradrenaline.

Results

Neither blood pressure nor heart rate changed in the different groups during the operative procedure. Basal values ranged from 99 ± 3 mmHg to 130 ± 5 mmHg and 379 ± 22 beats/min to 462 ± 11 beats/min, respectively.

Bilateral microinjection of 23 nmol (±)-α-methylnoradrenaline into the area of the NTS at the level of the obex caused a decrease of blood pressure and heart rate (Figure 1). Blood pressure and heart rate started to fall immediately after completion of the injections and reached a maximum of −33 ± 6 mmHg after 5 min and −66 ± 7 beats/min after 10 min, respectively. After 30 min, blood pressure was still significantly different from its control value ($P < 0.05$). A representative track into the area of the NTS is indicated in a cross section of the medulla oblongata at the level of the obex (Figure 2). Data from animals in which tracks were located more laterally or more medially were not used for this group.

Unilateral injection of 23 nmol α-methylnoradrenaline also caused a lowering in blood pressure and heart rate (maximal responses −22 ± 5 mmHg and −59 ± 15 beats/min, respectively, $n = 8$). In comparison with the responses after bilateral injections, these effects were less marked ($P < 0.05$ for the effect on blood pressure) and both blood pressure and heart rate returned to basal values within 20 minutes.

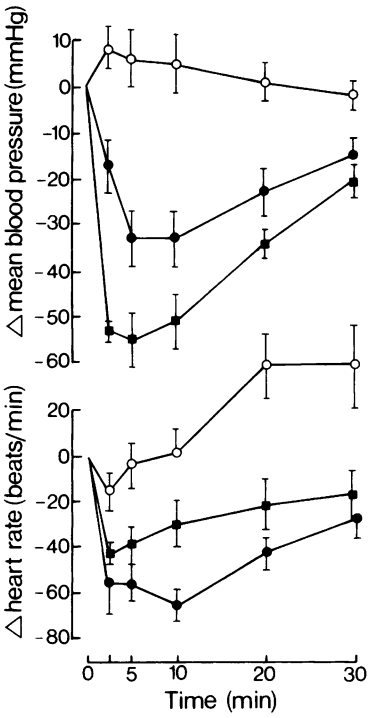

Figure 1 Effect of bilateral injection of α-methylnoradrenaline (23 nmol) into the area of the nucleus tractus solitarii on mean blood pressure and heart rate of anaesthetized rats and of vagotomized rats treated with atropine sulphate (5 mg/kg i.p.); (○) saline ($n = 9$); (●) α-methylnoradrenaline ($n = 6$); (■) α-methylnoradrenaline after vagotomy and atropine ($n = 6$).

In order to determine the effective localization in the NTS, the dose of 23 nmol α-methylnoradrenaline was applied in sixteen different sites of the medulla oblongata (Figure 3). The maximal blood pressure lowering effect occurred just caudal to the obex level, 0.5 mm lateral to the midline (−36 ± 1 mmHg). However, between 0.5 mm rostral and 1.0 mm caudal to the obex a similar but less pronounced effect on blood pressure was observed. The fall in blood pressure was associated with decreases in heart rate. All effective sites for the inhibitory cardiovascular effects were located in the NTS.

In order to see whether adrenaline and noradrenaline also decrease blood pressure after injection into the NTS a study was undertaken to compare the cardiovascular effects of three different doses (5.8, 23, 92 nmol) of these catecholamines (Figure 4). The effects on blood pressure were dose-dependent, except for the highest dose of noradrenaline (92 nmol) which caused a maximal decrease of blood pressure of only −19 ± 7 mmHg.

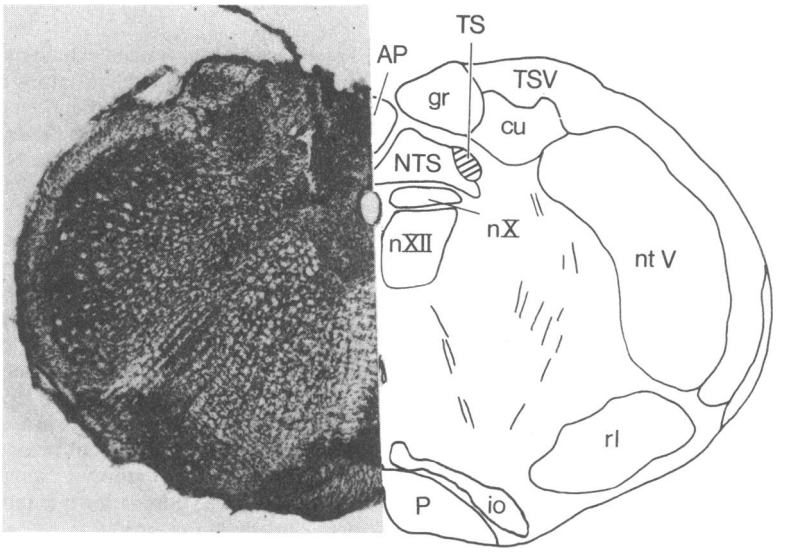

Figure 2 Cross section of the medulla oblongata at the level of the obex with photographic (left) reflection of tracks in the area of the nucleus tractus solitarii (0.5 mm lateral, 1 mm depth). Schematic representation of structures is shown in the right half of the figure. Abbreviations are according to Palkowitz & Jacobowitz (1974): AP=area postrema; cu=nucleus cuneatus; gr=nucleus gracilis; io=nucleus olivaris inferior; NTS=nucleus tractus solitarii; rl=nucleus reticularis; ntV=nucleus tractus spinalis nervi trigemini; nX=nucleus originis dorsalis vagi; nXII=nucleus originis nervi hypoglossi; P=tractus cortico spinalis; TS=tractus solitarius; TSV=tractus spinalis nervi trigemini.

This was a significantly smaller decrease ($P<0.05$) than that following 92 nmol α-methylnoradrenaline (−43 ± 4 mmHg) or adrenaline (−36 ± 3 mmHg). The maximal decrease in blood pressure after administration of adrenaline and noradrenaline was reached after 2.5 to 5 minutes. However, for α-methylnoradrenaline, the maximal response was not reached until 5 to 10 minutes. A similar pattern was observed for the effects on heart rate.

In order to study the effect of α-adrenoceptor blockade on the cardiovascular effects of α-methylnoradrenaline, the α-adrenoceptor antagonist, phentolamine, was injected bilaterally into the area of the NTS in a dose of 23 nmol, 10 min before the local

Table 1 Effect of bilateral injection of α-methylnoradrenaline (23 nmol) into the area of the nucleus tractus solitarii on mean blood pressure and heart rate of anaesthetized rats. Either saline or phentolamine (23 nmol) was injected bilaterally into the same site 10 min earlier

		Pretreatment		*Pretreatment*	
		Saline	*Phentolamine*	*Saline*	*Phentolamine*
Time (min) after injection of α-methylnoradrenaline		*Blood pressure (mmHg)*		*Heart rate (beats/min)*	
−10.0 (basal values)		127 ± 6 (10)	115 ± 4 (8)	379 ± 22	420 ± 22
0		123 ± 7	121 ± 7	357 ± 17	343 ± 22
1.0	Change with respect to time 0 values	−11 ± 5	+36 ± 6**	−39 ± 18	+36 ± 12*
2.5		−20 ± 4	+28 ± 6**	−27 ± 16	+31 ± 15*
5.0		−33 ± 4	+14 ± 7**	−19 ± 19	+31 ± 19
10.0		−30 ± 4	+8 ± 5**	−7 ± 24	+43 ± 24
20.0		−21 ± 4	+3 ± 7*	+23 ± 24	+69 ± 20
30.0		−13 ± 4	+4 ± 8	+44 ± 21	+78 ± 19

* $P<0.05$; ** $P<0.01$; compared to corresponding control value (saline pretreatment). Number of animals in parentheses.

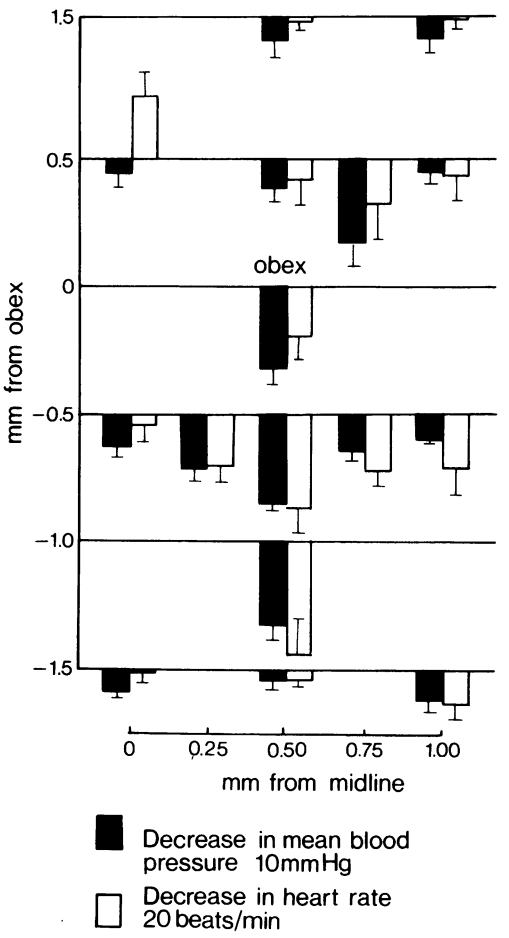

Figure 3 Topographical distribution in the medulla oblongata of maximal mean blood pressure and heart rate responses to 23 nmol α-methylnoradrenaline injected in sixteen different sites. Except in the midline, all injections were given bilaterally, ($n=5$ to 11).

injection of 23 nmol α-methylnoradrenaline. After phentolamine, the fall in blood pressure and heart rate was prevented and even reversed to an increase, the maximum occurring within approximately 2 min (Table 1).

Following bilateral vagotomy and intraperitoneal injection of atropine sulphate (5 mg/kg), 23 nmol α-methylnoradrenaline caused a decrease in blood pressure with a maximum of −55 mmHg at 5 min after injection (Figure 1). After 30 min, blood pressure was still significantly different ($P<0.01$) from the group treated with saline only. The decrease in blood pressure after the parasympathetic blockade seems to be intensified and to reach its maximum effect faster. In contrast, the decrease in heart rate was reduced by 35% in the pretreated group.

Discussion

These results confirm and extend our previous findings that α-methylnoradrenaline causes a lowering of blood pressure and heart rate after local injection into the area of the NTS (Nijkamp & De Jong, 1975; De Jong *et al.*, 1975a). Only in part of the NTS directly around the obex were these effects observed. This area of the NTS has a high density of catecholaminergic terminals as visualized histochemically (Fuxe, 1965). Mimicking of the effects of the endogenous neurotransmitter noradrenaline by α-methylnoradrenaline in this part of the brain seems a possibility, since noradrenaline also causes a fall in blood pressure after local application (De Jong, 1974; De Jong, Zandberg & Bohus, 1975b). However, α-methylnoradrenaline was more effective than noradrenaline. Because the responses to α-methylnoradrenaline were more prolonged than those to noradrenaline or adrenaline it is tempting to speculate that α-methyldopa produces its inhibitory effects on the cardiovascular system through α-methylnoradrenaline which, in the central nervous system, may be a more effective neurotransmitter on noradrenaline receptor sites. α-Adrenoceptors seem to be involved since phentolamine blocked the inhibitory effects and even reversed these effects to an initial increase in blood pressure and heart rate. This finding is in agreement with the inhibitory effect of intraventricularly injected phentolamine in rats on the blood pressure lowering effect of α-methyldopa (Finch & Haeusler, 1973). The effects on cardiovascular functions exerted in the NTS are stereospecific since (+)-noradrenaline was ineffective (De Jong & Nijkamp, 1975) and (+)-α-methylnoradrenaline in doses up to 200 nmol also failed to induce changes in blood pressure and heart rate (P. Zandberg & W. de Jong, unpublished observations). It is therefore likely that the difference in potency between (−)-α-methylnoradrenaline and (−)-noradrenaline is even greater than the difference between the racemic mixture of α-methylnoradrenaline and (−)-noradrenaline used in this study.

The increase in blood pressure and heart rate observed after α-blockade might be caused by an effect on β-adrenoceptors since α-methylnoradrenaline possesses β-agonistic activity and intraventricular injection of β-mimetics such as isoprenaline sometimes causes pressor responses and tachycardia (Toda, Matsuda & Shimamoto, 1969; Satchell, Freeman & Hopkins, 1971; Bhargava, Mishra & Tangri, 1972; Day & Roach, 1974).

After atropine and vagotomy the decrease in blood pressure after local injection of α-methylnoradrenaline into the area of the NTS is augmented. In unanaesthetized dogs, atropine antagonizes the blood pressure increase following intraventricular injection of acetylcholine into the brain (Lang & Rush, 1973; Laubie, 1975) while the hypertensive response is

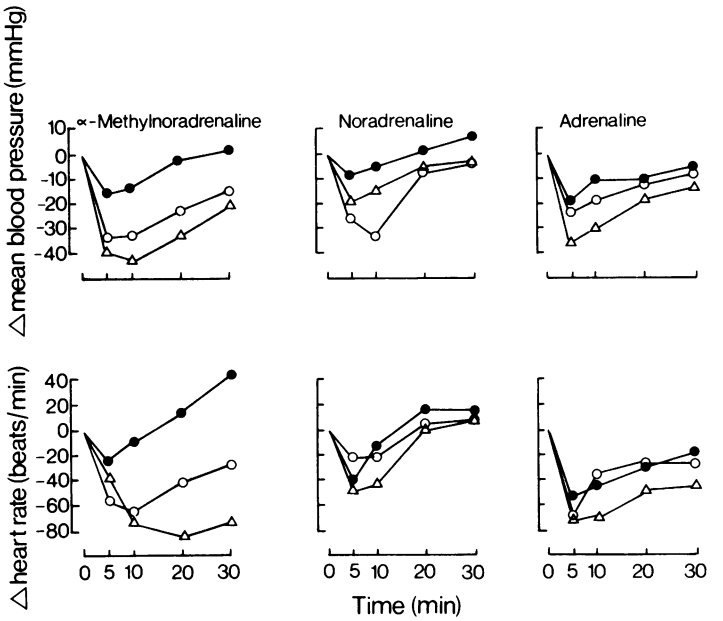

Figure 4 Effect of different doses of α-methylnoradrenaline, noradrenaline and adrenaline on mean blood pressure and heart rate of anaesthetized rats, ($n=4$ to 9): (●) 5.8 nmol; (○) 23.0 nmol; (△) 92.0 nmol.

potentiated by physostigmine. We found a dose-dependent increase in blood pressure after local injection of 1 and 5 μg physostigmine into the area of the NTS (unpublished results). So the more pronounced decrease in blood pressure by local injection of α-methylnoradrenaline after atropine and vagotomy might be explained by an inhibition of the activation of muscarinic receptors. In fact, this inhibitory effect of the cholinergic system does not seem to be restricted to the hypotensive action of α-methylnoradrenaline since Laubie (1975) reported that in unanaesthetized dogs, stimulation of muscarinic receptors counteracted the decrease in blood pressure brought about by the centrally acting anti-hypertensive agent clonidine (Schmitt, Schmitt, Fenard & Laubie, 1973; Schmitt, 1975), while vertebral artery injection of atropine allowed the effect to reappear.

It has been shown by Palkovitz & Jacobowitz (1974) that a reasonable amount of acetylcholinesterase activity is present just under the NTS. A neurotransmitter function for acetylcholine in this part of the brain, mediating the stimulating properties of α-methylnoradrenaline, therefore, seems possible. On this basis, it cannot be excluded that the anti-hypertensive agent α-methyldopa acting through its metabolite α-methylnoradrenaline may exert two opposing effects; an increase of blood pressure may be caused via a cholinergic synapse as well as a decrease of blood pressure mediated via noradrenergic synapse.

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References

BHARGAVA, K.P., MISHRA, N. & TANGRI, K.K. (1972). An analysis of central adrenoceptors for control of cardiovascular function. *Br. J. Pharmac.*, **45**, 596–602.

DAY, M.D., ROACH, A.G. & WHITING, R.L. (1973). The mechanism of the antihypertensive action of α-methyldopa in hypertensive rats. *Eur. J. Pharmac.*, **21**, 271–280.

DAY, M.D. & ROACH, A.G. (1974). Central α- and β-adrenoceptors modifying arterial blood pressure and heart rate in conscious cats. *Br. J. Pharmac.*, **51**, 325–333.

DE JONG, W. (1974). Noradrenaline: central inhibitory control of blood pressure and heart rate. *Eur. J. Pharmac.*, **29**, 179–181.

DE JONG, W. & NIJKAMP, F.P. (1975). Hypotensive action of noradrenaline and α-methylnoradrenaline in the area of the nucleus tractus solitarii in the rat brain stem. In *Central Action of Drugs in Blood Pressure Regulation,* ed. Davies, D.S. & Reid, J.L. pp. 179–180. London: Pitman Publishing Ltd.

DE JONG, W., NIJKAMP, F.P. & BOHUS, B. (1975a). Role of noradrenaline and serotonin in the central control of blood pressure in normotensive and spontaneously hypertensive rats. *Archs Int. Pharmacodyn. Ther.,* **213**, 272–284.

DE JONG, W., ZANDBERG, P. & BOHUS, B. (1975b). Central inhibitory noradrenergic cardiovascular control. *Progress in Brain Research,* **42**, 285–298.

FINCH, L. & HAEUSLER, G. (1973). Further evidence for a central hypotensive action of β-methyldopa in both the rat and the cat. *Br. J. Pharmac.,* **47**, 217–228.

FINCH, L., HERSOM, A. & HICKS, P. (1975). Studies on the hypotensive action of α-methyldopamine. *Br. J. Pharmac.,* **54**, 445–451.

FUXE, K. (1965). Evidence for the existence of monoamine neurons in the central nervous system. IV. The distributions of monoamine nerve terminals in the central nervous system. *Acta physiol. scand.,* **64**, Suppl. 247, 39–85.

HEISE, A. & KRONEBERG, G. (1972). α-Sympathetic receptor stimulation in the brain and hypotensive activity of α-methyldopa. *Eur. J. Pharmac.,* **17**, 315–317.

HENNING, M. (1975). Central sympathetic transmitters and hypertension. *Clin. Sci. Mol. Med.,* **48**, 195s–203s.

HENNING, M. & RUBENSON, R. (1971). Evidence that the hypotensive action of methyldopa is mediated by central actions of methylnoradrenaline. *J. Pharm. Pharmac.,* **23**, 407–411.

HENNING, M. & VAN ZWIETEN, P.A. (1968). Central hypotensive effect of α-methyldopa. *J. Pharm. Pharmac.,* **20**, 409–417.

LANG, W.J. & RUSH, M.L. (1973). Cardiovascular responses to injections of cholinergic drugs into the cerebral ventricles of unanaesthetised dogs. *Br. J. Pharmac.,* **47**, 196–205.

LAUBIE, M. (1975). Pharmacological evidence for interactions of cholinergic and noradrenergic mechanism in central cardiovascular control. *Proceedings of the Sixth Int. Congr. of Pharmac. (Helsinki), Drug Therapy,* **4**, 79–85.

NIJKAMP, F.P., EZER, J. & DE JONG, W. (1975). Central inhibitory effects of α-methyldopa on blood pressure, heart rate and body temperature of renal hypertensive rats. *Eur. J. Pharmac.,* **31**, 243–249.

NIJKAMP, F.P. & DE JONG, W. (1975). α-Methylnoradrenaline induced hypotension and bradycardia after administration into the area of the nucleus tractus solitarii. *Eur. J. Pharmac.,* **32**, 361–364.

PALKOVITZ, M. & JACOBOWITZ, D.M. (1974). Topographic atlas of catecholamine and acetylcholinesterase-containing neurons in the rat brain. II. Hindbrain (Mesencephalon, rhombencephalon). *J. comp. Neur.,* **157**, 29–42.

SATCHELL, D.G., FREEMAN, S.E. & HOPKINS, S.V. (1971). Effects of α-methylnoradrenaline on cardiac metabolism. *Biochem. Pharmac.,* **20**, 1691–1694.

SCHMITT, H. (1975). On some unexplained effect of clonidine, In *Recent Advances in Hypertension II,* ed. Milliez, P. & Safar, M. pp. 63–74, Monaco: Boehringer and Ingelheim.

SCHMITT, H., SCHMITT, H., FENARD, S. & LAUBIE, M. (1973). Evidence for a sympathomimetic component inhibiting the sympathetic centres: Nature of the receptor. In *Symposium on Pharmacological Agents and Biogenic Amines in the Central Nervous System.* ed. Knoll, J. & Magyar, K. pp. 177–194, Budapest, Akademiai Kiado.

TODA, N., MATSUDA, Y. & SHIMAMOTO, K. (1969). Cardiovascular effects of sympathomimetic amines injected into the cerebral ventricles of rabbits *Int. J. Neuropharmac.,* **8**, 451–461.

VAN ZWIETEN, P.A. (1975). Antihypertensive drugs with a central action. *Progr. Pharmac.,* **1**, 1–63.

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