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EFFECTS OF NORADNAMINE (5-AMINOMETHYL-2,3,7,8-TETRAHYDROXYDIBENZO-[a,e[CYCLOHEPTATRIENE) ON THE BLOOD PRESSURE OF THE ANAESTHETIZED CAT AND RAT

BY

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It has been proposed (Roberts & Broadley, 1965) that noradnamine (5-aminomethyl-2,3,7,8-tetrahydroxydibenzo-[a,e]cycloheptatriene) might be formed under certain conditions *in vivo* from noradrenaline. Although the original purpose of the proposal was to offer some common hypothesis for the modes of action of the various drugs used in the treatment of depression, the postulated route of synthesis of noradnamine from noradrenaline is equally applicable to enzyme mechanisms outside of the central nervous system. We have been also interested, therefore, in the peripheral pharmacology of noradnamine. We now wish to report our preliminary findings on blood pressure responses in the anaesthetized cat and rat.

METHODS

Cats of either sex, 1.2 to 3.3 kg, were anaesthetized with chloralose, 7.5 ml./kg of a 1% w/v solution in 0.9% w/v saline injected into a femoral venous cannula, after induction with ether. Male or female rats, 150 to 500 g, were anaesthetized with 25% w/v urethane (0.6 ml./100 g) or pentobarbitone sodium (Nembutal, Abbott Laboratories, 0.1 ml./100 g) intraperitoneally. Carotid arterial blood pressures were measured with mercury manometers, and drugs were injected *via* cannulae in femoral (cats) or external jugular (rats) veins. Heparin (1,000 u./kg) was given intravenously.

In most experiments control submaximal responses to intravenously injected noradrenaline, adrenaline, isoprenaline, tyramine, tetramethylammonium, histamine and acetylcholine were obtained before the intravenous administration of noradrenaline. In some experiments, using cats, blood pressure was recorded from a femoral artery and contractions of the nictitating membrane in response to stimulation (5V, 5–20 pulses/sec of 2 msec duration for 30 sec to maximal effect) of the pre- or post-ganglionic cervical sympathetic nerve were recorded using a frontal writing lever (15 times magnification, 3 g tension). Blood pressure changes in response to stimulation of the descending vagus nerve were also periodically recorded in some cats.

Drugs

(−)-Adrenaline acid tartrate, (±)-isoprenaline sulphate (Burroughs Wellcome & Co.), (−)-noradrenaline acid tartrate (Koch-Light Laboratories Ltd.), tyramine hydrochloride, tetramethyl-

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ammonium chloride, histamine acid phosphate and acetylcholine chloride (B.D.H. Ltd.) were all obtained commercially. Noradnamine hydrochloride was synthesized in our own laboratory (Roberts & Broadley, 1967). All doses in the text are expressed in terms of the appropriate base.

RESULTS

Blood pressure of anaesthetized rat

Intravenous administration of noradnamine (120 μg/kg) produced a rise in blood pressure. This response increased in size with increase in dose to a maximum (600 μg/kg); further increases in dose resulted in progressively smaller responses until doses of 12 mg/kg produced falls in blood pressure (Fig. 1).

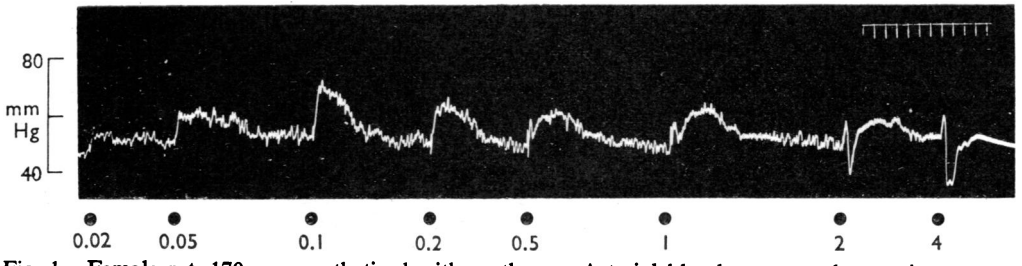

Fig. 1. Female rat, 170 g, anaesthetized with urethane. Arterial blood pressure changes in response to increasing intravenous doses (mg) of noradnamine. Time trace in min.

The initial pressor responses were abolished following the administration of cocaine at a dose level (5 mg/kg intraperitoneally) which reduced the pressor response to injected tyramine and augmented that due to injected noradrenaline or adrenaline (Fig. 2).

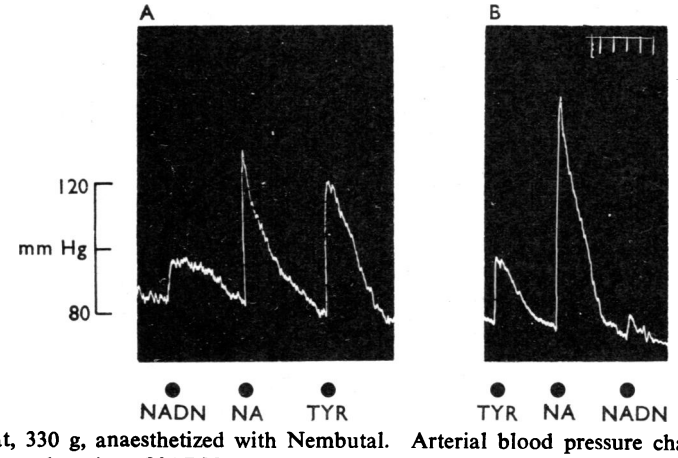

Fig. 2. Male rat, 330 g, anaesthetized with Nembutal. Arterial blood pressure changes in response to 50 μg noradnamine (NADN), 0.1 μg noradrenaline (NA), and 50 μg tyramine (TYR) administered intravenously before (A) and $\frac{1}{2}$ hr after (B) 5 mg/kg cocaine intraperitoneally. Time trace in min.

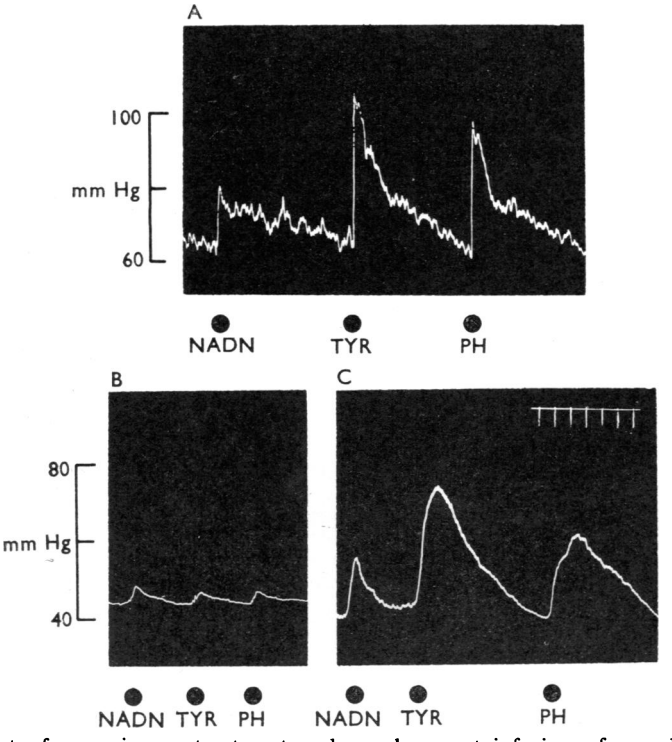

Fig. 3. The effect of reserpine pretreatment and a subsequent infusion of noradrenaline on the responses of the rat blood pressure (urethane anaesthesia) to intravenous injections of 50 μg noradnamine (NADN), 50 μg tyramine (TYR) and 50 μg β-phenylethylamine (PH). Panel A, control male rat 290 g. Panel B, reserpinized (5 mg/kg intraperitoneally 24 hr and 48 hr before the experiment) male rat 250 g, and Panel C, the same reserpinized rat following the intravenous infusion of 10 μg noradrenaline over 30 min. Time trace in min.

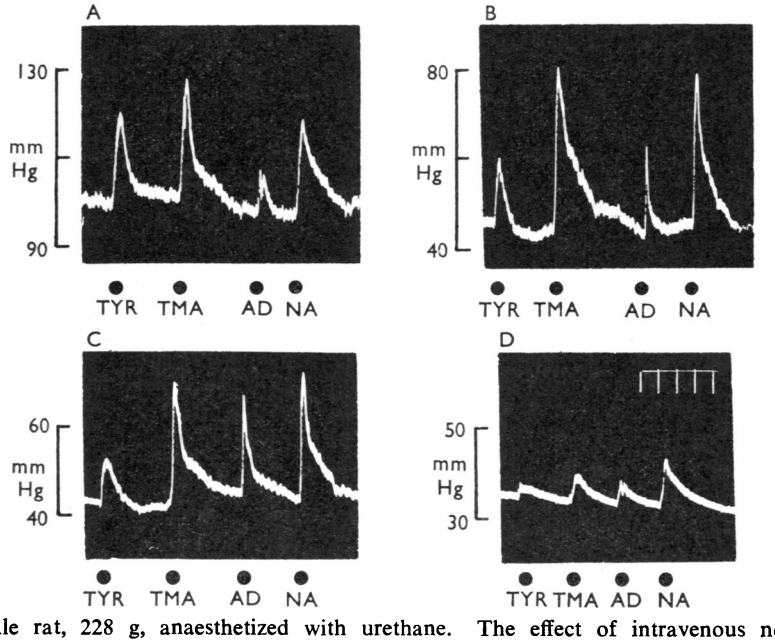

Fig. 4. Male rat, 228 g, anaesthetized with urethane. The effect of intravenous noradnamine (between A and B 80 μg, between B and C 40 μg, between C and D 4 mg) on the blood pressure responses to intravenous injections of 50 μg tyramine (TYR), 5 μg tetramethylammonium chloride (TMA), 0.2 μg adrenaline (AD) and 0.2 μg noradrenaline (NA). Time trace in min.

Intravenous administration of noradnamine did not cause a rise in blood pressure in rats pretreated with reserpine (5 mg/kg intraperitoneally) 24 and 48 hr before use, but an infusion of noradrenaline (40 μg/kg, 20 μg/hr) restored the response (Fig. 3).

When the reference drugs (see Methods) were injected following doses of noradnamine totalling 350 μg/kg the responses to tyramine were reduced, while those to noradrenaline, adrenaline and tetramethylammonium were potentiated (Fig. 4A and B). Responses to the other reference drugs were unaffected. After further doses of noradnamine the

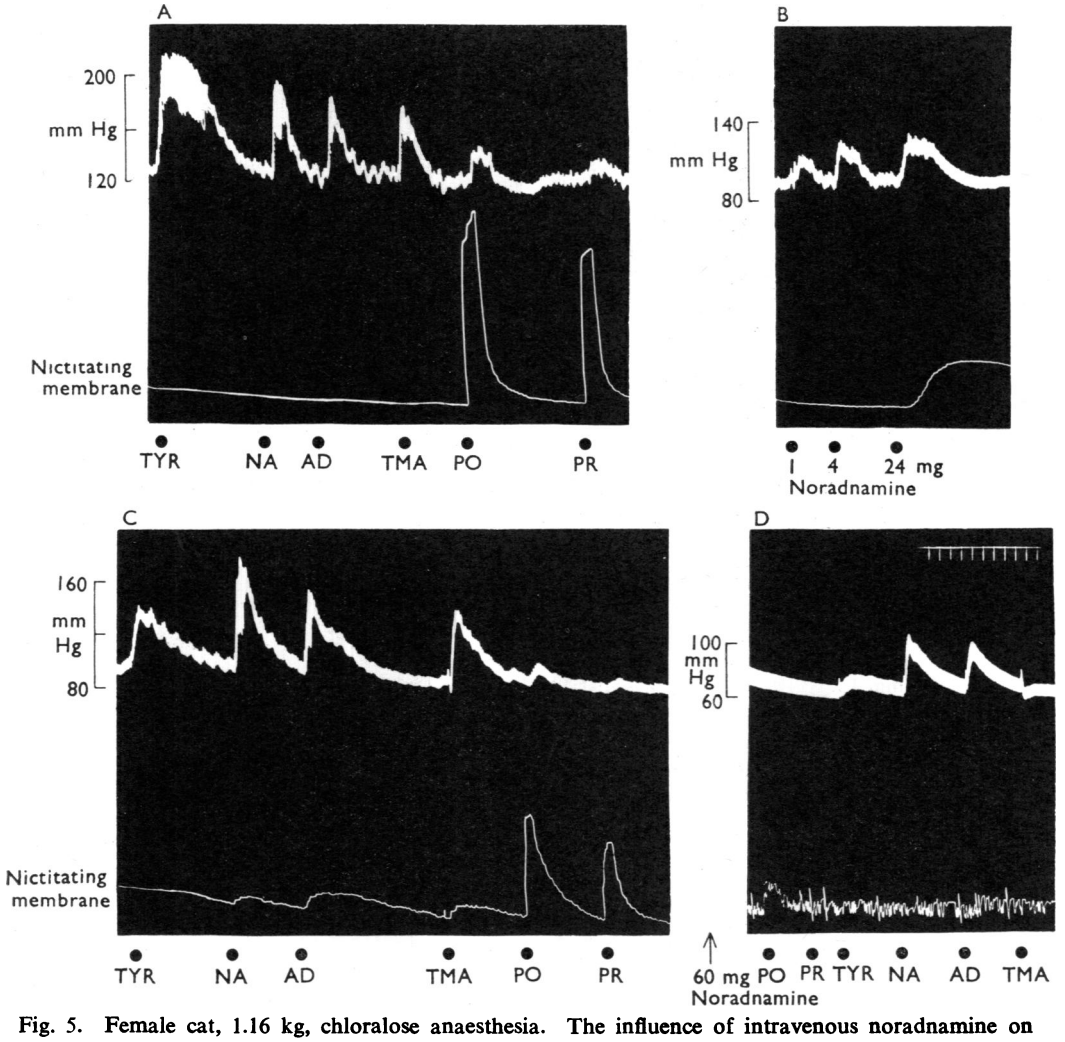

Fig. 5. Female cat, 1.16 kg, chloralose anaesthesia. The influence of intravenous noradnamine on the responses of the blood pressure and nictitating membrane to 450 μg tyramine (TYR), 1 μg noradrenaline (NA), 2 μg adrenaline (AD) and 40 μg tetramethylammonium chloride (TMA) injected intravenously and to pre-ganglionic (PR) and post-ganglionic (PO) stimulation (5 V, 5 pulses/sec of 2 msec duration to maximum response) of the ascending cervical sympathetic nerve. Between A and C, 29 mg noradnamine (B) and between C and D, 60 mg noradnamine was given. Time trace in min.

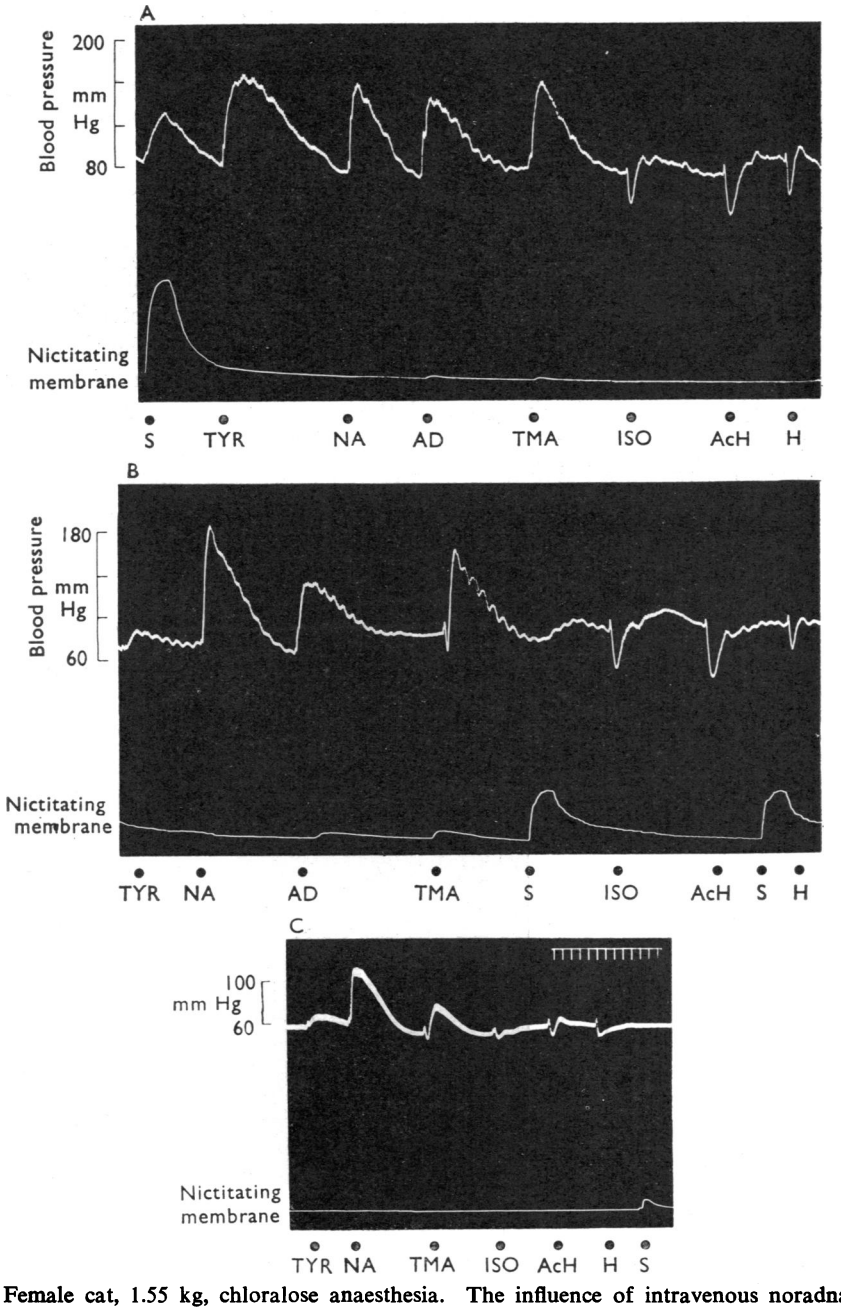

Fig. 6. Female cat, 1.55 kg, chloralose anaesthesia. The influence of intravenous noradnamine on the responses of the blood pressure and nictitating membrane to pre-ganglionic stimulation (5V, 20 pulses/sec of 2 msec duration to maximum effect) of the ascending cervical sympathetic nerve (S) and to intravenous injections of 450 μg tyramine (TYR), 2 μg noradrenaline (NOR), 2 μg adrenaline (AD), 50 μg tetramethylammonium chloride (TMA), 2 μg isoprenaline (ISO), 1 μg acetylcholine (AcH) and 1 μg histamine (H). Between A and B 25 mg/kg noradnamine and between B and C 35 mg/kg noradnamine injected intravenously. Time trace in min.

pressor responses first to tetramethylammonium, and then to noradrenaline and adrenaline were also reduced (Fig. 4C and D). The responses to isoprenaline, acetylcholine and histamine were unaffected by noradnamine at the lower dose levels, but were reduced following the higher doses.

Blood pressure and nictitating membrane of anaesthetized cat

Similar results to those already described for the rat were obtained when the experiments were repeated on cats (Figs. 5 and 6). In addition, doses of noradnamine (20 mg/kg) that prevented the pressor responses to tyramine, yet potentiated those to noradrenaline, adrenaline and tetramethylammonium, also inhibited the responses of the nictitating membrane to both pre- and post-ganglionic stimulation of the ascending cervical nerve (Fig. 5A and C) and reduced the pressor response to occlusion of the carotid arteries (Fig. 7). By contrast, even large doses of noradnamine had little effect on the fall in blood pressure induced by stimulation of the descending vagus nerve.

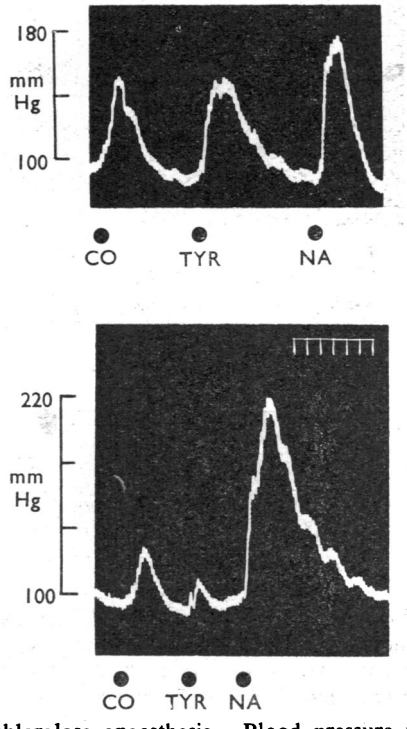

Fig. 7. Female cat, 3.3 kg, chloralose anaesthesia. Blood pressure responses to bilateral carotid occlusion for 1 min (CO), 200 μg tyramine (TYR) and 1 μg noradrenaline (NA) before (top trace) and after (bottom trace), 20 mg/kg noradnamine injected intravenously. Time trace in min.

The apparent anomaly of the blood pressure and nictitating membrane responses to tetramethylammonium, which are mediated *via* stimulation of sympathetic ganglia, being potentiated and not blocked by doses of noradnamine which inhibited the responses of

tissues to sympathetic nerve stimulation was clarified by performing bilateral adrenalectomy during some of the experiments. This procedure completely abolished the responses to tetramethylammonium in the presence of noradnamine (40 mg/kg) on both the blood pressure and the nictitating membrane (Fig. 8).

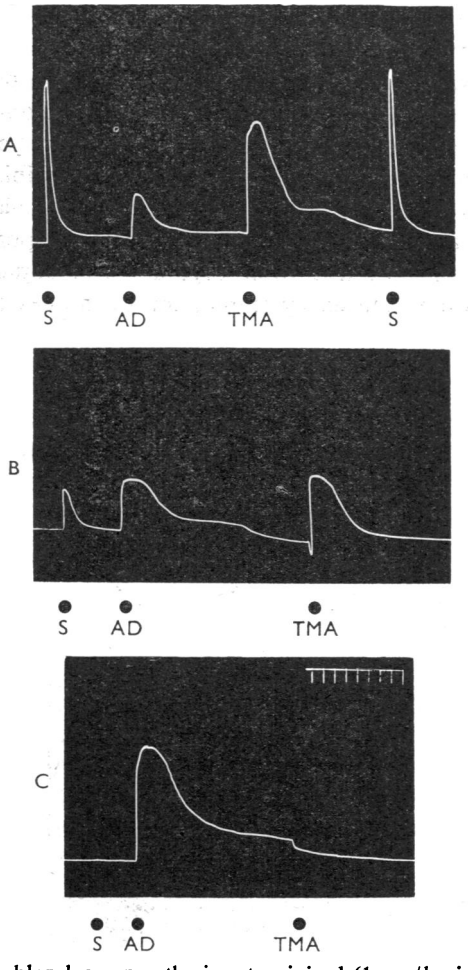

Fig. 8. Male cat, 3.16 kg, chloralose anaesthesia, atropinized (1 mg/kg intraperitoneally). Responses of the nictitating membrane to post-ganglionic stimulation (5V, 5 pulses/sec of 2 msec duration to maximal effect) of the ascending cervical sympathetic nerve (S), 20 μg adrenaline (AD) and 1 mg tetramethylammonium chloride (TMA). Between A and B 40 mg/kg noradnamine administered intravenously and between B and C bilateral adrenalectomy performed. Time trace in min.

DISCUSSION

The pharmacological actions of noradnamine on the cardiovascular system appear to be exclusively concerned with adrenergic mechanisms.

We were at first worried by the initial sympathomimetic action of noradnamine

because of possible contamination with small amounts of noradrenaline from the synthesis (Roberts & Broadley, 1967). That the pressor responses are abolished and not potentiated in the presence of cocaine, and their absence in reserpinized animals, however, provides satisfactory evidence that the sympathomimetic action of noradnamine, like that of tyramine, is an indirect one resulting from the release of catecholamines from storage sites (Tainter & Chang, 1927 ; Burn & Rand, 1958 ; Muscholl, 1966).

By contrast the inhibition of tyramine responses and potentiation of adrenaline and noradrenaline responses following the administration of noradnamine itself appear to be associated with the prevention of release of amines rather than uptake. Cocaine, which abolishes the sympathomimetic action of tyramine and potentiates that of injected noradrenaline by inhibiting their uptake into storage sites (Muscholl, 1961), also inhibits the usual re-uptake of nerve released noradrenaline into sympathetic nerve endings. Consequently the responses of tissues to sympathetic nerve stimulation are increased in the presence of cocaine (Trendelenburg, 1959). Doses of noradnamine that reduce the pressor response to tyramine, however, also reduce the responses of the nictitating membrane to nervous stimulation.

Furthermore, since the contractions of the nictitating membrane are reduced in response to post-ganglionic stimulation as readily as they are to pre-ganglionic stimulation, the depressant action is peripheral to the ganglion. A further contrast between noradnamine and ganglion blocking agent is that the pressor response to the ganglion stimulating drug tetramethylammonium is increased rather than impaired after administration of noradnamine. This, together with the abolition of the tetramethylammonium response following adrenalectomy, indicates that the release of pressor amines from the adrenal medulla is not interfered with by noradnamine. The increased response to chemical stimulation of the medulla in this way may be accounted for by an enhancement of the effect of the released adrenaline and noradrenaline. Similarly, the fact that the responses to intravenously administered sympathomimetic catecholamines are also enhanced, as they are after sympathectomy (Langer & Trendelenburg, 1966), indicates that the block of the nictitating membrane responses mediated by the ascending cervical sympathetic nerve is not due to antagonism of adrenaline or noradrenaline. Local anaesthesia also appears not to be implicated in this blocking action, since the effects of stimulating the cholinergic vagus nerve to the heart as measured on the arterial blood pressure were unaffected.

The pharmacological actions so far described are similar to those exhibited by drugs such as xylocholine, guanethidine and bretylium (Boura & Green, 1965) and allow us to classify noradnamine as an adrenergic neurone blocking agent. Although this type of activity is in itself sufficient to explain the potentiation of the responses of pressor amines either injected intravenously or released from the adrenal medulla, recent investigations have shown that noradnamine is an inhibitor of catechol-O-methyl transferase (Abbs, Broadley & Roberts, 1967). Inhibition of this enzyme is associated with enhancement of the responses of sympathomimetic amines (Wylie, Archer & Arnold, 1961).

SUMMARY

1. The influence of noradnamine on certain adrenergic mechanisms in the cat and the rat have been investigated.

2. Noradnamine administered intravenously in both species produced pressor responses which were absent following pretreatment with reserpine or cocaine. The sympathomimetic actions of noradnamine were therefore considered to result solely from the release of endogenous catecholamines.

3. Doses of noradnamine which themselves abolished the pressor responses to tyramine and potentiated those to adrenaline and noradrenaline also inhibited the responses of the cat nictitating membrane to both pre- and post-ganglionic stimulation. By contrast the release of amines from the adrenal medulla in response to the ganglion stimulant tetramethylammonium chloride was unaffected.

4. Effects on cholinergic or histaminic mechanisms were not evident at dose levels of noradnamine producing the above effects on adrenergic mechanisms.

5. Noradnamine was accordingly classified as an adrenergic neurone blocking agent.

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