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PHARMACOLOGICAL EXPERIMENTS ON THE RELEASE OF THE SYMPATHETIC TRANSMITTER

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The effects of various pharmacological agents on the release of noradrenaline from sympathetic nerve endings have been described by Boyd, Chang & Rand (1960) and by Brandon & Rand (1961). These authors used the contractile response of the effector organ as an index of the amount of transmitter liberated. Interpretation of their results is, however, difficult because not only are we ignorant of the relation between transmitter liberation and effector response, but, as Vane (1962) has pointed out, many drugs alter non-specifically the tissue response to catechol amines. We have therefore done our experiment by collecting venous blood from the spleen of the cat and estimating the noradrenaline liberated by stimulation of the nerves.

Cocaine, anticholinesterases and hexamethonium do not appear to affect the liberation of noradrenaline by the nerves or its uptake by the tissues of the spleen.

METHODS

Cats were anaesthetized with ethyl chloride, ether and intravenous chloralose 80 mg/kg. The spleen, its nerve supply, arteries and venous drainage were prepared as described by Brown & Gillespie (1957), except that instead of tying the splenic nerve early in the preparation, we left ligation as late as possible in order to prevent those effects of neuronal rest that may appear after 1–2 hr (Brown, Davies & Ferry, 1961). In some experiments the spleen was perfused with the cat's own blood through a constant-output perfusion pump. This consisted of a tube of silicone rubber compressed by three plates. The two outer plates were input and output valves, the larger, middle or ventricular plate compressed most of the tubing between the valves. The valves and ventricles were operated by pulses of air produced in three coupled compressors which ran at 90 c/min. The output of the pump could be varied by altering the stroke volume. In most experiments the stomach and duodenum were removed in order to gain better access to the arterial supply of the spleen. Blood was taken from the cat through a polythene cannula placed in the stump of the superior mesenteric artery and, after passing through the pump, was ejected into the coeliac axis through a cannula placed in the hepatic artery. The coeliac axis was tied between the aorta and the hepatic artery after the pump had been connected and had begun to pump blood into the coeliac axis; there was thus no interruption of the blood supply to the spleen. Experiments showed that, if the splenic blood supply was interrupted, the resistance to

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flow was greatly increased when the perfusion eventually began, presumably through the shutting down of part of the splenic vascular bed. The ventricle and valves were sufficiently small to be inserted into the abdomen together with all the tubing containing blood. The abdomen was filled with liquid paraffin kept at 37° C by a heater. The blood pressure on the output side of the pump was recorded either with a mercury manometer or with an Elema Schönander electromanometer. When samples of the splenic venous blood were being taken, the venous pressure remained constant and therefore the rise in pressure on the arterial input side of the spleen represented the change in the resistance to flow offered by the splenic blood vessels and gave a measure of the response of the spleen to nerve stimulation. The preparation and the original model of the pump (Blakeley, Ferry, Parsons & Schuster, 1961) and an improved version of the pump have been demonstrated to the Physiological Society (Blakeley, Brown, Dickson & Ferry, 1962).

Intravenous injections were made into the right external jugular vein. Close arterial injections into the spleen were made via the hepatic or left gastric artery, the site depending on whether or not the preparation was artificially perfused.

Acetylcholine injections were prepared as described by Ferry (1963). Hexamethonium bromide and eserine sulphate were dissolved in 0·9 % NaCl; Dibenyline (phenoxybenzamine) was first dissolved in alcohol and then injected as a fine suspension in 0·9 % NaCl. The other blocking agent used was Hydergine (Sandoz); this is a preparation of dihydroergocornine, dihydroergocristine and dihydroergokryptine.

RESULTS

The effect of anticholinesterases on the output of noradrenaline

The adrenergic blocking agents phenoxybenzamine, phentolamine and Hydergine greatly increase the output of noradrenaline from the spleen when the splenic nerves are stimulated (Brown & Gillespie, 1957; Brown *et al.* 1961). The explanation for this effect suggested by these authors is that the blocking agent prevents the uptake by the splenic tissues of the liberated transmitter. An alternative hypothesis has been proposed by Boyd *et al.* (1960), who found anticholinesterase activity in some adrenergic blocking agents and suggested that their effect in increasing noradrenaline output was due to their action upon a cholinergic link in the sympathetic post-ganglionic pathway.

We have accordingly tested the effects of the powerful anticholinesterases eserine and prostigmine on the output of noradrenaline from the spleen and compared it with the effect of an adrenergic blocking agent. Figure 1 shows the results of one such experiment. Excitation of the splenic post-ganglionic nerves with maximal shocks at a frequency of 10/sec evoked the usual small overflow of transmitter. Administration of eserine sulphate 0·3 mg/kg by intravenous injection, a dose sufficient to cause vigorous skeletal muscular twitching, was without effect on the transmitter overflow. A subsequent intravenous dose of Hydergine produced a fourfold increase in overflow. A similar experiment in which neostigmine methylsulphate was given in a dose of 0·1 mg/kg gave identical results. This appears to us to provide conclusive evidence that the

effect of the adrenergic blocking agents in increasing the output of transmitter is not attributable to any anticholinesterase properties they may possess.

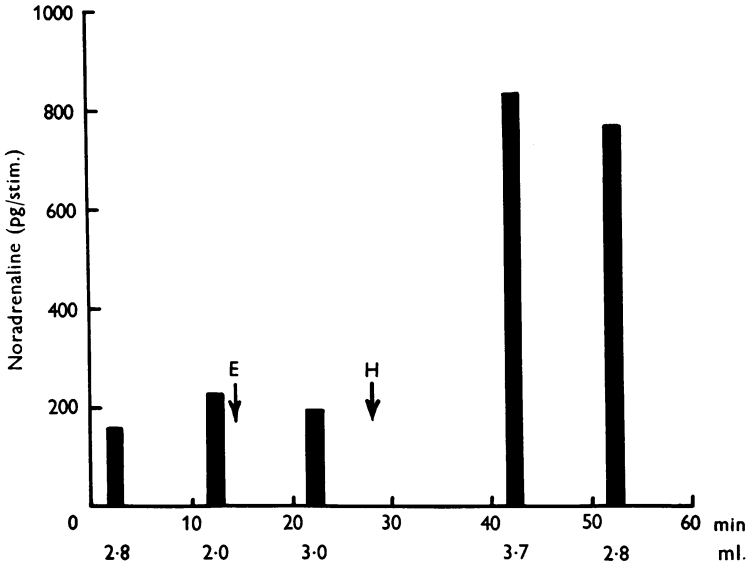

Fig. 1. Cat 2·3 kg; 1 mg atropine. Output of noradrenaline from spleen after groups of 200 maximal stimuli to splenic nerve at 10/sec. Collection time 40 sec; at E, eserine 0·3 mg/kg intravenously; at H, Hydergine 0·5 mg/kg. In this and subsequent figures the values below each block show the plasma volume of the sample.

The effects of hexamethonium

The sympathomimetic effects in the spleen of arterially injected acetylcholine are abolished by hexamethonium (Ferry, 1963), but hexamethonium has no effect upon the motor response of the spleen of the dog to stimulation of the splenic nerves (Daly & Scott, 1961). It seems, therefore, *a priori* unlikely that there exists any cholinergic link sensitive to hexamethonium in the sympathetic post-ganglionic supply of the spleen. It appeared desirable, however, to ascertain the effect of hexamethonium, in ganglion-blocking doses, on the output of sympathetic transmitter; because, as we have already pointed out, too little is yet known about the relation between transmitter output and tissue response.

Liberation of noradrenaline by acetylcholine. The arterial injection of acetylcholine causes a liberation of noradrenaline (Brandon & Rand, 1961; Brandon & Boyd, 1962) and a contraction of the spleen. This liberation of noradrenaline is not affected by atropine, but is abolished by hexamethonium (Fig. 2).

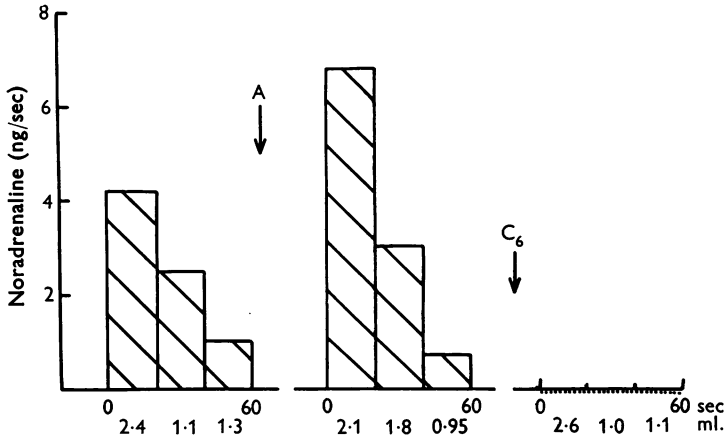

Fig. 2. Rate of liberation of noradrenaline by acetylcholine, and effect of hexamethonium. Cat 2·1 kg, phenoxybenzamine 10 mg/kg. Output of noradrenaline after three injections of 1 mg acetylcholine into spleen via hepatic artery. At A, 1 mg atropine; at C_6, 10 mg/kg hexamethonium intravenously.

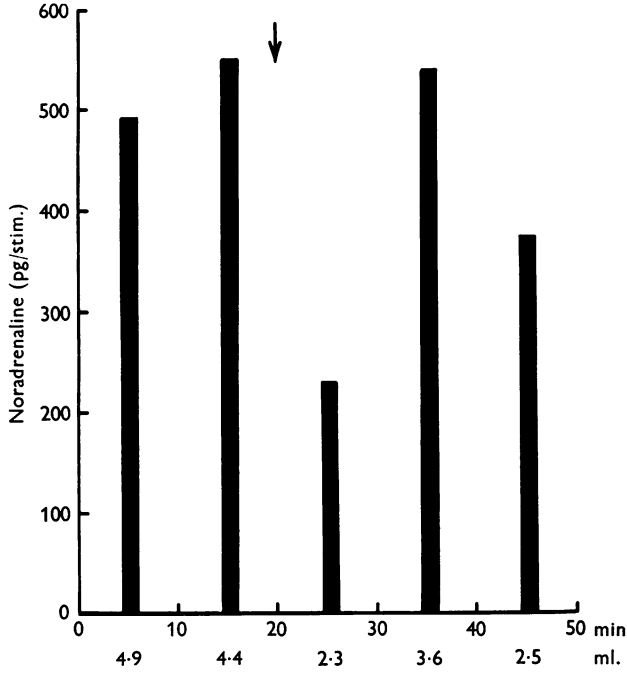

Fig. 3. Cat 3·7 kg; pump perfusion of spleen. Output of noradrenaline after groups of 200 maximal stimuli to splenic nerve at 30/sec. At arrow, 10 mg/kg hexamethonium intravenously. For details see text.

Effect on output of transmitter. In our first experiments on the effects of hexamethonium on the output of transmitter with nerve stimulation we were unable to secure consistent results, the output sometimes being increased and at other times diminished. If hexamethonium in sufficient dose is given intravenously, it inevitably leads to a fall of general blood pressure and a decrease in the circulation through the spleen. As Brown & Gillespie (1957) pointed out, the output of transmitter in the splenic venous blood is to some extent dependent upon blood flow, and a great

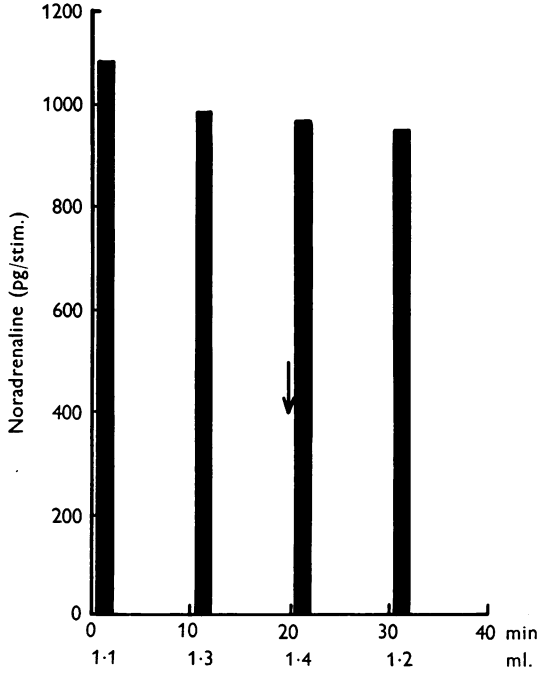

Fig. 4. Cat 2·7 kg; phenoxybenzamine 10 mg/kg intravenously; pump perfusion of spleen. Output of noradrenaline with groups of 200 stimuli at 30/sec. At arrow, hexamethonium 10 mg/kg.

diminution in the plasma volume of a sample is often associated with a fall of transmitter content. Figure 3 illustrates this point. The spleen was perfused with the constant-output pump, but, in spite of this, after the injection of hexamethonium there was a fall in the plasma volume and in the noradrenaline content of the sample. The fall in plasma volume was due to a decrease in the blood flow through the pump, attributable to the general circulatory disturbance produced by the hexamethonium. The next control sample showed an increased blood flow, a normal plasma volume and an output of transmitter identical with that of the previous control sample. During the final sampling period, the pump output was

intentionally reduced; the sample had a smaller volume and contained less transmitter.

If precautions are taken to maintain the flow through the spleen constant, hexamethonium does not reduce the transmitter output at 10 or 30/sec, with or without a blocking agent. We have, indeed, some reason to think that at the lower frequency, hexamethonium may actually increase transmitter liberation and overflow (Figs. 4, 5, 6). Brown, Davies &

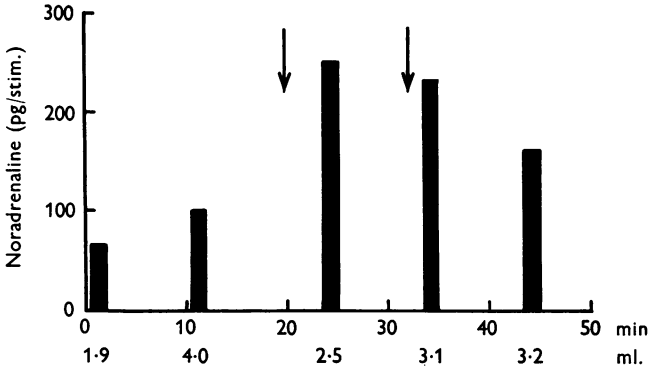

Fig. 5. Cat 1·7 kg; pump perfusion of spleen. Output of noradrenaline with groups of 200 stimuli at 10/sec. At arrows, successive intravenous injections of hexamethonium 10 mg/kg.

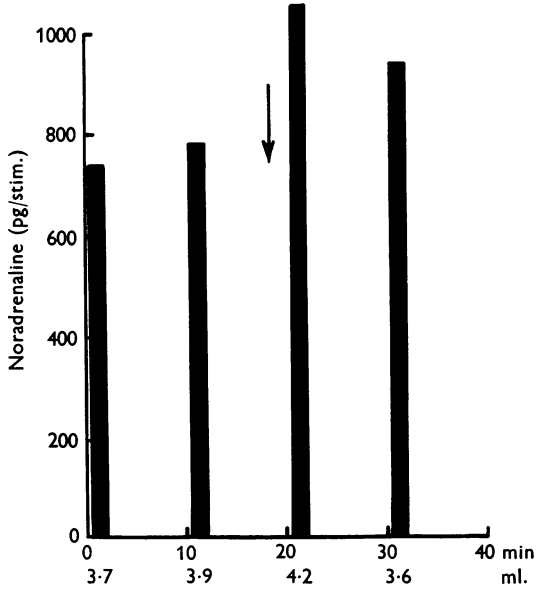

Fig. 6. Cat 2·7 kg; phenoxybenzamine 10 mg/kg; pump perfusion of spleen. Output of noradrenaline with groups of 200 stimuli at 10/sec. At arrow, intravenous hexamethonium 10 mg/kg.

Gillespie (1958) similarly concluded that hexamethonium did not depress transmitter liberation in the small intestine.

The response of the spleen to nerve stimulation. The records of the output pressure of the pump show the change in the resistance to flow through the spleen. The experiment in Fig. 7 illustrates the effect of intravenous hexamethonium on the output of transmitter and on the response of the spleen. The records of pump output pressure show that there was little change in the response of the spleen. These results confirm the findings of Daly & Scott (1961) on the dog.

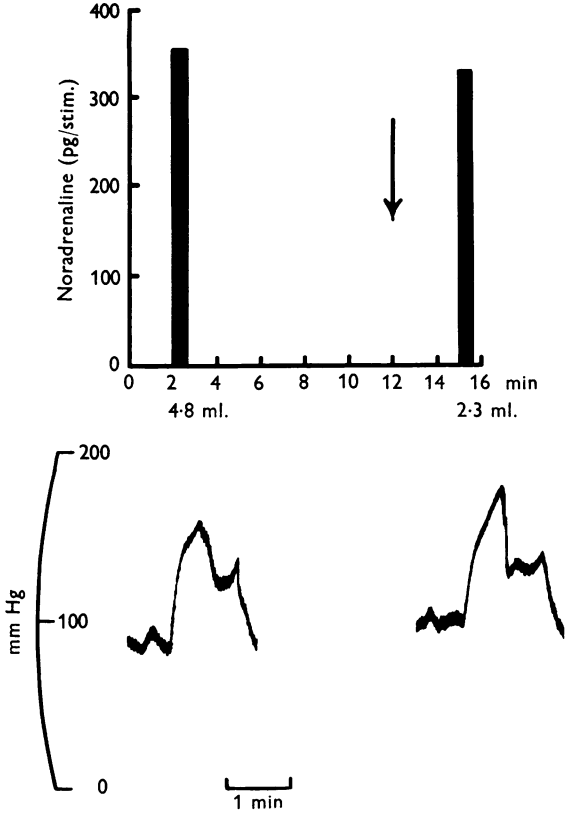

Fig. 7. Cat 3·1 kg. Upper record, output of noradrenaline from 2 successive groups of 200 stimuli at 10/sec; at arrow, hexamethonium 10 mg/kg intravenously. Lower record, output pressure of pump during collection period.

The effect of cocaine on the uptake and release of transmitter

Trendelenburg (1959) found that, although cocaine prolonged the retention of injected catechol amines in the plasma, the output of transmitter from the spleen of the cat at 30/sec (11 experiments) and at 10/sec (1 experiment) was not changed by the drug. We have extended these

experiments, using stimulation at 10/sec because the output at this frequency is low and capable of elevation by blocking agents, whereas the output at 30/sec cannot be raised very much. We have investigated the effect of intravenous cocaine 5 mg/kg on two cats and in both experiments the results were almost identical. The results of one of these are shown in Fig. 8. Cocaine does not affect the output of noradrenaline, but the administration of Hydergine raises the output to the expected level. We have concluded from this experiment that cocaine affects neither the uptake nor the liberation (Brown *et al.* 1961) of transmitter.

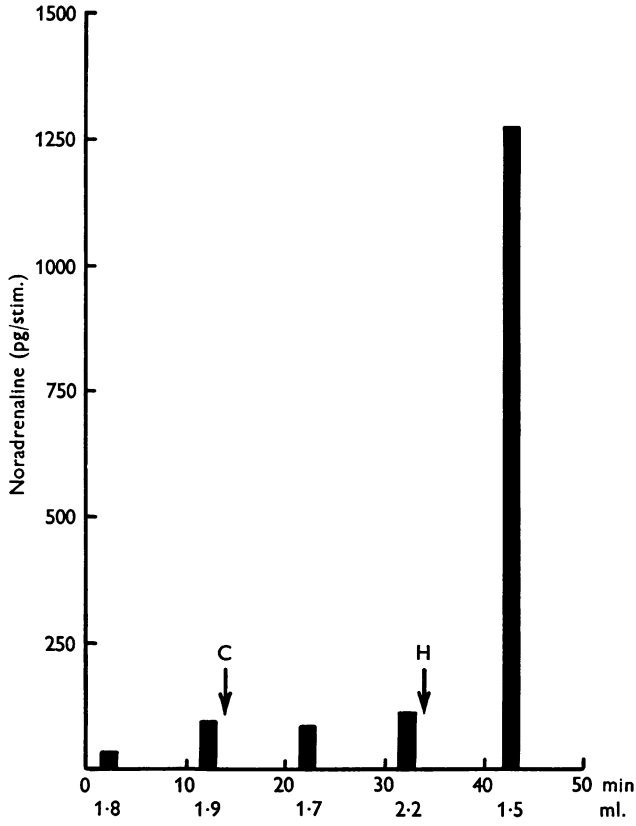

Fig. 8. Cat 2·1 kg. Output of noradrenaline from spleen with groups of 200 stimuli at 10/sec. At C, cocaine hydrochloride 5 mg/kg and at H, Hydergine 0·5 mg/kg intravenously.

DISCUSSION

Our experiments have given further evidence of the unique property possessed by adrenergic blocking agents of raising the output of the sympathetic transmitter in the venous blood from the spleen when the

splenic nerves are excited. It is surprising that cocaine both in Trendelenburg's hands and ours had so little effect. It is evident that the conditions affecting the disappearance from the systemic blood of injected catechol amines are quite different from those affecting the entry into the venous blood of the transmitter liberated within the tissues by nervous impulses.

The absence of effect of hexamethonium was to be expected, in view of its failure to alter the tissue response to nerve stimulation. The importance of our finding is in relation to the effects of artificially injected acetylcholine. Hexamethonium prevents the excitation by acetylcholine of sensory nerves (Douglas & Gray, 1953; Diamond, 1959) and also of sympathetic post-ganglionic C fibres (Ferry, 1963). The sympathomimetic effects on the spleen of injected acetylcholine have been used as evidence by Brandon & Rand (1961) to support the idea of the existence of a cholinergic link in the sympathetic post-ganglionic pathway. It seems clear now that acetylcholine owes its effects to an excitation of motor fibres, leading in turn to a liberation of noradrenaline and a consequent contraction of the spleen. The liberation of noradrenaline by acetylcholine is abolished by hexamethonium in doses that leave quite unaffected the motor response of the spleen when its nerves are stimulated. The liberation and uptake of noradrenaline by nerve impulses are similarly not diminished by hexamethonium, in doses sufficient to suppress the effects of injected acetylcholine. The sympathomimetic effects of injected acetylcholine cannot, therefore, be regarded as evidence for a cholinergic link in the sympathetic post-ganglionic pathway.

Our experiments with anticholinesterases have been equally conclusive. Injections of eserine and prostigmine in doses large enough profoundly to affect skeletal neuromuscular transmission did not alter the output of noradrenaline, although the subsequent administration of an adrenergic blocking agent increased it by a factor of four. We feel justified in concluding that the adrenergic blocking agents do not owe their effects to any anticholinesterase activity they may happen to possess.

SUMMARY

1. Eserine, neostigmine and cocaine do not affect the liberation by the nerves or the uptake of the sympathetic transmitter in the spleen of cats under chloralose anaesthesia.

2. The release of noradrenaline by arterial injection of large amounts of acetylcholine was prevented by hexamethonium.

3. Hexamethonium did not affect the motor response of the splenic blood vessels to nerve stimulation and had no effect on the output of transmitter.

4. These results suggest a different interpretation of some of the evidence advanced in support of a cholinergic link in the post-ganglionic sympathetic adrenergic neuro-effector pathway.

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