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POTENTIATION OF THE PRESSOR ACTION OF NORADRENALINE BY HEXAMETHONIUM, TETRAETHYLAMMONIUM, AND METHANTHELINE

BY

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The pressor action of intravenous adrenaline, noradrenaline, angiotonin, and renin is greatly increased after ganglionic transmission is blocked by tetraethylammonium (Corcoran and Page, 1947; Page and Taylor, 1947, 1950; Moe, 1947; Page, 1949). This pharmacological effect provides a useful clinical tool in the diagnosis of phaeochromocytoma and in the therapy of the serious hypotensive conditions that can follow the administration of ganglion-blocking drugs (La Due, Murison, and Pack, 1948).

The phenomenon has been explained in different and sometimes opposite ways. It was ascribed to a peripheral sensitization by chemical denervation, or to an anti-amine-oxidase activity of tetraethylammonium in the tissues (Page and Taylor, 1947). Later on, the same authors (Page, 1949; Page and Taylor, 1950) considered as a mechanism the release from the liver of a noradrenaline-like substance. The effect could not be produced when the spinal cord segments lower than C6 were destroyed, but it was still present when the spinal cord was simply transected at a high level (Page, 1949; Page and Taylor, 1950).

The latter observation was confirmed by Saint Clair and Stone (1951) and is inconsistent with the hypothesis, suggested by Moe (1947), that the potentiation is due to paralysis of the normal nervous mechanism compensating any haemodynamic modification. Were this hypothesis true, the simple severance of the spinal cord at C1 should prevent the appearance of the potentiation.

The potentiation occurs also after vagotomy or atropine administration. This observation does not support the hypothesis of Rosa and Grassi (1949–50) that the potentiation is due to the blockade of a parasympathetic reflex vasodilatation.

Our experiments were directed toward a further investigation of this controversial problem. We used different drugs and tested them in relation to a single vasoactive substance, (−)-noradrenaline. Besides tetraethylammonium, we have studied hexamethonium, which is without some of tetraethylammonium's secondary effects, and methantheline (" Banthine "), since after its administration the parasympathetic blockade occurs at an earlier stage than the sympathetic.

METHODS

Fifty-six adult dogs anaesthetized with chloralose (70–100 mg./kg.) were used. Blood pressure was recorded from a carotid or femoral artery by a Hg manometer. To obtain a steady hypertension, (−)-noradrenaline (8 to 24 mg./l.) was infused intravenously, at a rate differing from experiment to experiment and dependent on the hypertensive level desired. This hypertension corresponds haemodynamically to the pressor outbursts of phaeochromocytoma. When the blood pressure had reached steady values, ganglion-blocking drugs were injected intravenously.

The following substances were used: hexamethonium bitartrate (0.05 to 6.0 mg./kg.), tetraethylammonium bromide (5.0 to 18.0 mg./kg.), and methantheline bromide (0.06 to 7.0 mg./kg.). Hexamethonium doses are all given in terms of the base, tetraethylammonium and methantheline doses in terms of the salts.

RESULTS

Potentiation of Noradrenaline Hypertension.— Thirty of a total of 31 dogs were subjected to bilateral cervical vagotomy before testing the vascular responses; hexamethonium was used in 15 animals, methantheline in 8, and tetraethylammonium in 8.

In 12 dogs, during an infusion of noradrenaline, an increase of blood pressure occurred after administration of hexamethonium in amounts larger than 2.5 mg./kg.; however 4.0 mg./kg. or more was often required to obtain more constant and clear-cut pressor effects (up to 50–60 mm. Hg).

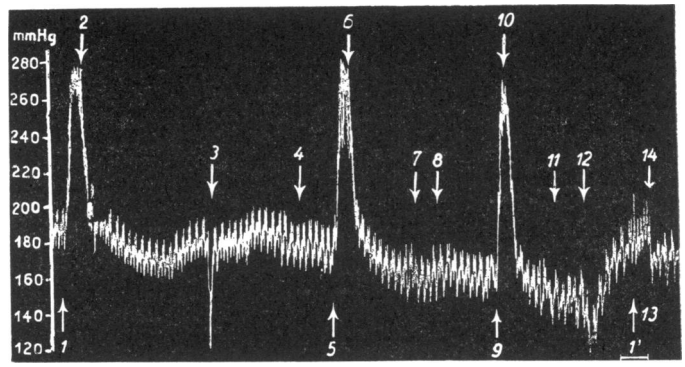

FIG. 1.—Dog, chloralose, vagi cut. Record of arterial blood pressure. Methantheline bromide, i.v., 0.06 mg./kg. at 4, 0.25 mg./kg. at 8, and 3.0 mg./kg. at 12. Peripheral end of right vagus stimulated at 3, 7, and 11. Carotid arteries occluded between 1–2, 5–6, 9–10, and 13–14. (−)-Noradrenaline was infused i.v. to produce a sustained rise of blood pressure throughout the experiment.

The potentiation could be produced several times in the same animal by injecting the effective doses, even at short intervals of time. Doses less than 2.0 mg./kg. did not produce potentiation, even when administered in close succession, but the phenomenon could be observed if the doses were progressively increased. In 3 animals no potentiation was obtained; in 2 of them the drug was administered in insufficient amount, whereas in the third experiment the effect did not occur in spite of large doses and repeated administration.

In 8 vagotomized animals, in which methantheline was injected, the pressor effect of noradrenaline was clearly augmented. The potentiation developed often, but not constantly, after a short-lasting hypotension (Fig. 1). The threshold effect was obtained with 3.0 mg./kg. of the drug. Further increase of blood pressure could be elicited by repeated administration of effective doses, even when a following injection occurred shortly after a previous one (Fig. 2). Subthreshold doses were ineffective even when administered repeatedly.

In 6 to 8 animals, tetraethylammonium bromide potentiated the noradrenaline hypertension. As for hexamethonium and methantheline, repeated administration of effective doses (5.0 to 18.0 mg./kg.) elicited a further hypertension, whereas the close repetition of subthreshold amounts was constantly ineffective. The potentiation could not be observed in two experiments, in spite of the injection of high doses of the drug, which usually produce the phenomenon in other animals.

Thus the three substances studied potentiate the pressor effect of noradrenaline. Generally, the stronger the pressor effect the longer is its duration. The maximal duration was 4–5 min. for tetraethylammonium and hexamethonium, and 7 min. for methantheline. We gained the impression that the magnitude of the potentiation does not depend on the hypertensive level established by infusion of noradrenaline. Of course, this holds only within a given range of pressure values, that is when the peripheral blood vessels can respond to the drug with a further constriction.

Baroceptive Mechanisms.—The potentiation could be ascribed to the suppression, by the drugs, of the baroceptive reflexes which counteract the peripheral vasopressive influence of noradrenaline. A blockade of the vagal cardio-inhibitory pathways, or of the efferent autonomic paths regulating the vasomotor tone, is likely to prevent the regulatory influence of both carotid sinus and cardio-aortic reflexes. That the first mechanism alone is not responsible for the potentiation is shown by the well-known fact, which we have been able to confirm in our experiments, that potentiation is still obtainable in bilaterally vagotomized animals. The second mechanism is supported by the fact that the increase of blood pressure elicited by bilateral clamping of the common carotid (the so-called carotid sinus reflex) is

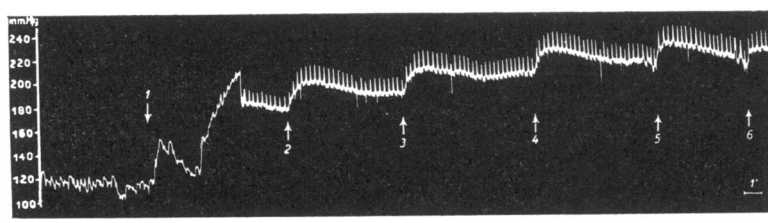

FIG. 2.—Dog, chloralose, vagi cut. (−)-Noradrenaline infused continuously i.v. at rate of 3.4 mg./kg./min. from 1. At 2, 4, 5, and 6, 6.0 mg./kg. methantheline bromide i.v., and at 3, 4.0 mg./kg. methantheline bromide i.v.

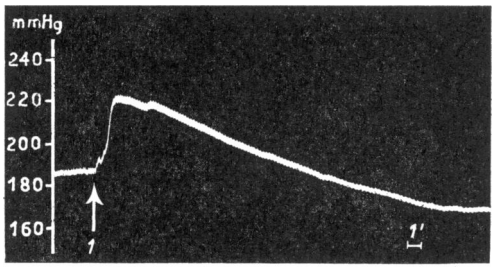

FIG. 3.—Dog, chloralose, vagi cut and spinal cord ablated from C1 to L2. Continuous i.v. (−)-noradrenaline, at a rate of 5.0 mg./kg./min. At 1, tetraethylammonium bromide 15.0 mg./kg. i.v.

completely abolished by these drugs and with the same doses which give potentiation. Actually, as soon as the influence of the carotid sinus baroceptors on the vasomotor component of the circulation is abolished, the potentiation is observed. These observations seem to support the hypothesis that blockade of baroceptive regulation and potentiation are causally related phenomena.

Serious difficulties are, however, shown by our methantheline experiments. With this drug, the fall of blood pressure elicited by faradizing the

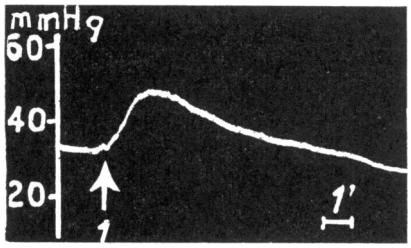

FIG. 4.—Dog, chloralose, vagi cut and spinal cord ablated from C1 to L2. Noradrenaline was *not* infused. At 1, tetraethylammonium bromide 15.0 mg./kg. i.v.

peripheral end of the vagus nerve is blocked for doses (0.06 mg./kg.) about 50 times smaller than those which are necessary for blocking the carotid sinus reflexes (Fig. 1). And yet the potentiation is obtained only when these reflexes are abolished (Fig. 1, observations 12, 13, and 14). Hence there is no doubt that potentiation is not due to blockade of the cardio-inhibitory component of the baroceptive reflexes. A vasomotor compensation by these reflexes of the peripheral action of noradrenaline might occur either through an increase of vasodilator activity or through a lowering of vasoconstrictor tone. It is unlikely that, for doses of methantheline about 50 times higher than those which block the vagal inhibition, the parasympathetic pathways are still active. If this assumption, which, however, cannot be regarded as crucially proved, is accepted, the conclusion should be drawn that the drug acts by preventing the peripheral effect of a baroceptive decrease of the central vasomotor tone. This appears to be an unlikely assumption, however, since the peripheral vasomotor tone was controlled by the continuous noradrenaline infusion, and is disproved, moreover, by the experiments reported below.

Supraspinal and Spinal Mechanisms.—If the suppression of the normal nervous mechanisms of circulatory homeostasis were the cause of potentiation, this would be absent in the spinal preparation after bilateral vagotomy. That this is not so, at least for tetraethylammonium, has been clearly demonstrated by Page and Taylor (1950) and Saint Clair and Stone (1951), who observed an increased pressor effect of noradrenaline injections following administration of tetraethylammonium in both normal and spinal animals. However, it is well known that tetraethylammonium has, besides a ganglion-blocking action, a pressor effect the origin of which is not clear.

It appeared worth while, therefore, to test the effect also of hexamethonium and methantheline in spinal (section at C1) vagotomized dogs, during continuous noradrenaline infusion. In 7 spinal dogs the potentiation occurred at the same doses, and with the same duration and intensity, as in normal animals. The phenomenon was not modified by the integrity or the section of the vagi.

To test the importance of the spinal cord in potentiation, we have carried out total ablation of the spinal cord, from C1 to L2, in 12 vagotomized animals. In 5 of these, to which tetraethyl-

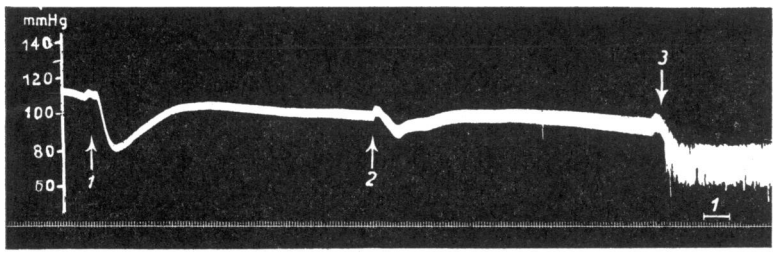

FIG. 5.—Dog, chloralose, vagi cut and spinal cord ablated from C1 to L2. Moderate hypertension was caused by continuous intravenous infusion of (−)-noradrenaline. At 1, 2, and 3, hexamethonium bitartrate 5.0 mg./kg. i.v.

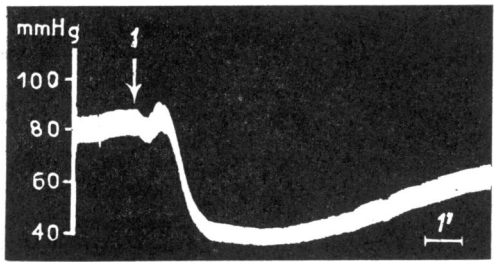

FIG. 6.—Dog, preparation as in Fig. 5. At 1, 4.0 mg./kg. methantheline bromide i.v.

ammonium was injected, a constant hypertensive effect was obtained (Fig. 3), which, however, occurred even when no noradrenaline was infused (Fig. 4). In the other 7 experiments, in which hexamethonium and methantheline were used, the administration of these drugs constantly elicited a blood-pressure decrease. Hence, the potentiation by these drugs was abolished by ablation of the cord (Figs. 5 and 6).

DISCUSSION

The potentiation of the pressor effect of noradrenaline by the injection of tetraethylammonium (Corcoran and Page, 1947 ; Page and Taylor, 1947 and 1950 ; Moe, 1947 ; Page, 1949) and of hexamethonium (Paton, 1951) is confirmed and the same phenomenon can be elicited also by administration of methantheline.

Our experimental methods have differed from those used by most previous authors. They tested the effect of noradrenaline in normal conditions and after intravenous administration of tetraethylammonium. The results obtained by the latter method are open to three main criticisms: (i) data obtained in two different haemodynamic conditions (normal pressure and hypotension from ganglionic blockade) are compared ; (ii) the augmented pressor responses to noradrenaline could be simply a consequence of the lower blood-pressure level produced by injection of the blocking drug (this objection could be safely dismissed only if the absolute level of noradrenaline hypertension, after administration of ganglion-blocking drugs, were higher than in normal conditions) ; (iii) data obtained at some interval of time are compared, when various factors may have interfered with the effect. It is well known, for example, that the action of ganglion-blocking substances is subject to rapid modifications.

Our experimental procedure overcomes these objections, emphasizes the critical importance of the dose of the ganglion-blocking agent and, in particular, enables the production of closely succeeding, and even superimposed, potentiations. Furthermore, interruption of noradrenaline infusion in the animals with the spinal cord destroyed from C1 to L2 has provided clear evidence for a direct hypertensive action of tetraethylammonium. This effect had been observed by previous authors (Page, 1949). Finally, since hexamethonium and methantheline have not been shown to share such an action, the potentiation can be ascribed to the main common effect of the three substances—the ganglion-blocking activity. However, the use of tetraethylammonium in the study of the potentiation does not appear completely safe, because of its hypertensive side-effect. This is not likely to depend on an inhibition of amine oxidase, because it occurs, without infusion of noradrenaline, after the spinal cord is destroyed.

The evidence provided in our experiments does not support the hypothesis advanced by Moe (1947), that the potentiation is a consequence of the paralysis of the brain stem mechanisms concerned in circulatory homeostasis. Since the effect was abolished by ablating the spinal cord, from C1 to L2, these segments seem to be essential for the phenomenon of potentiation.

Only tetraethylammonium shows a hypertensive effect in animals with the spinal cord ablated, but this occurs without infusion of noradrenaline, and, therefore, it cannot be considered a potentiation. On the other hand, the hypothesis of a decreased vascular sensitivity in such animals, as a result of surgical shock, can be dismissed, since in the same animals very good responses were elicited by noradrenaline infusion.

The potentiation might occur in the spinal animal either by abolishing its vasodilator tone or by stimulating the preganglionic sympathetic neurones which are responsible for the spinal vasoconstrictor tone (Heymans *et al.*, 1936a, b, c ; Leriche and Fontaine, 1930 ; Bartorelli and Capretti, 1946).

The first hypothesis is only indirectly supported by Uvnäs' experiments, since we do not know if the cholinergic vasodilator outflow reported by the Swedish investigators is tonic in character.

We are at loss to understand how a stimulation of sympathetic preganglionic neurones (second hypothesis) might be effective, since the potentiation occurs, as shown by our carotid sinus experiments, just when the main efferent vasopressor paths are blocked.

It could be maintained that the spinal cord is actually not involved in the potentiation, which might be explained in terms of some purely peripheral effect such as a blockade of amine oxidase.

It must be conceded that when the spinal cord is transected at C1, or ablated, the production of noradrenaline and adrenaline, both as hormones and as transmitters, is likely to be so low that the influence of a blockade of the amine oxidase might easily be missed. It is difficult to understand, however, why injected noradrenaline should not be potentiated in these conditions. Our experiments have shown that in the preparations with the spinal cord ablated, and infused with noradrenaline, potentiation never occurs, and that a fall of blood pressure is elicited by injection of methantheline and hexamethonium. Further experiments are obviously required before a satisfactory explanation of the mechanism of potentiation can be given.

Our procedure is the experimental counterpart of the pathogenic and haemodynamic conditions associated with a phaeochromocytoma. Injections of ganglion-blocking drugs are used as a diagnostic test in man, a hypertensive response being considered as a positive sign of phaeochromocytoma. However, the results of the present experiments indicate that the potentiation occurs on administration of ganglion-blocking drugs in amounts far larger than those used in man for clinical purposes. Hence, the diagnostic value of the test is doubtful, unless it can be demonstrated that the sensitivity to ganglion-blocking agents is greater in man than in dogs, or that it is decreased by anaesthesia. Furthermore, the potentiation occurs only in the presence of a considerable amount of circulating noradrenaline. It would be advisable, therefore, to time the test during typical hypertensive outbursts, but this procedure would produce at least a temporary increase of the patient's subjective disturbances.

It might be convenient to aim the test at producing evidence not of a further hypertension but of the absence of hypotension. However, it has to be remembered that some hypertensive patients, without any adrenal medullary tumour, do not show hypotensive responses to the administration of ganglion-blocking drugs.

On the other hand, the use of the high doses which were effective in our experimental animals would expose the patient to the danger of an enormous fall of blood pressure, whenever the hypertensive condition was not due to a phaeochromocytoma. Serious danger could be avoided, however, if a noradrenaline solution were on hand to be infused intravenously (Bartorelli and Folli, 1954). We do not share the opinion of those authors (Page and Taylor, 1947) who think that the use of noradrenaline during deep hypotension from ganglion-blockade is contraindicated. We think that continuous intravenous infusion, drop by drop, with frequent blood-pressure measurements, permits the exact adjustment of the dose to the requirements of the patient; the necessary amount of noradrenaline is decreased by the potentiating effect of the ganglion-blocking drug.

Summary

1. The intravenous injection of hexamethonium (2.5 to 6.0 mg./kg.), methantheline (3.0 mg./kg. or more) and tetraethylammonium (5.0 to 18.0 mg./kg.) elicits an increase of blood pressure in animals into which (−)-noradrenaline is continuously infused.

2. The potentiation is not affected by vagotomy and section of the spinal cord at a high level.

3. The potentiation occurs only when both parasympathetic and sympathetic transmissions are blocked. Small doses of methantheline, which block only the parasympathetic innervation of the heart, are insufficient to produce potentiation.

4. Ablation of the spinal cord (from C1 to L2) prevents the potentiation; in these conditions tetraethylammonium still causes a rise of blood pressure, whether (−)-noradrenaline is infused or not. On the contrary, no blood-pressure increase results from the injection of hexamethonium and methantheline in such animals.

References

Bartorelli, C., and Capretti, G. (1946). *Boll. Soc. it. Biol. sp.*, **22**, 444.
—— and Folli, G. (1954). *Clin. Terap.* (in press).
Corcoran, A. C., and Page, I. H. (1947). *Proc. Soc. exp. Biol., N.Y.*, **66**, 148.
Heymans, C., Bouckaert, J. J., and Wierzuchowski, M. (1936a). *C.R. Soc. Biol., Paris*, **123**, 286.
—— —— (1936b). Ibid., **123**, 986.
—— —— Farber, S., and Hsu, F. Y. (1936c). Ibid., **122**, 115.
La Due, J. S., Murison, P. J., and Pack, G. T. (1948). *Ann. int. Med.*, **29**, 914.
Leriche, R., and Fontaine, R. (1930). *Presse méd.*, **38**, 1233.
Moe, G. K. (1947). *J. Amer. med. Ass.*, **137**, 1115.
Page, I. H. (1949). *Amer. J. Physiol.*, **158**, 403.
—— and Taylor, R. D. (1947). *J. Amer. med. Ass.*, **135**, **348**.
—— —— (1950). *Circulation*, **1**, 1233.
Paton, W. D. M. (1951). *Brit. med. J.*, **1**, 773.
Rosa, L., and Grassi, C. (1949–50). *Atti Soc. lombarda Sci. med. biol.*, **5**, 542.
Saint Clair, R. W., and Stone, C. A. (1951). *Proc. Soc. exp. Biol., N.Y.*, **77**, 542.
Uvnäs, B. (1954). *Physiol. Rev.*, **34**, 608.