THE INHIBITION OF ADRENALINE BY SPASMOLYTIC AGENTS

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It has long been known that[1] certain fibres in the autonomic nervous system which are classified on anatomical grounds as post-ganglionic sympathetic fibres are functionally cholinergic in nature. The effects of stimulation of such fibres are not reproduced by injection of adrenaline, but are potentiated by previous administration of eserine and are abolished by atropine sulphate. Bussel[2] has collected much evidence, from a review of earlier literature on the subject and from new experimentation, which supports the view that atropine has an inhibitory action upon the effects of stimulating post-ganglionic adrenergic sympathetic fibres and upon the actions of adrenaline itself. These investigations were carried out upon various preparations of portions of the vascular bed of animals and upon the contraction of the nictitating membrane in cats. It was found that in contrast to the behaviour of atropine, hyoscine hydrobromide did not modify the action of adrenaline on the vessels of the dog leg or on the nictitating membrane of the cat, or depress the level of the blood pressure in etherised cats in the doses administered.

The present paper reports upon an action of adrenaline which is inhibited by relatively small doses of atropine sulphate. A comparison was made between the effects of atropine sulphate (*r*), *l*-hyoscine, *l*-hyoscyamine, *l*-homatropine, trasentin 6H and 2786 R.P. (neoantergan) upon adrenaline activity in the preparation described below. The effect of neoantergan on the action of adrenaline on the isolated uterus of pregnant rabbit, and on the isolated gut segment of rabbit and guinea-pig was investigated. The effect of neo-antergan, atropine and trasentin 6H on adrenaline action on the blood pressure of spinal cats was also tested. In some cases comparison was made between the effects of the spasmolytic agents on the actions of *l*-adrenaline (B.P.) and *l-nor*-adrenaline.[3]

Graham[4] showed that the isolated duodenum of the duck or drake contracts on addition of adrenaline to the fluid in which the strip of gut is suspended. The tissue is stiff and shows little spontaneous movement. The ileum of the drake and all parts of the intestine of fowls and pigeons show more activity and relax on addition of adrenaline. As Barsoum and Gaddum[5] have shown, the rectal cæcum of the fowl is sensitive to adrenaline in a concentration of 10^{-9}. The bath in which the duck gut was suspended was kept at 38°C. and was of 100 ml. volume. Drugs were added in solution in 0·5 ml. of saline solution.

The Action of Adrenaline on the Isolated Duodenum of the Drake and Interference with it by Spasmolytic Agents

Adrenaline in a concentration of 2×10^{-8} or more causes a transient but powerful contraction of the isolated duodenum of ducks. This contraction is usually but not always followed by a period of relaxation which may or may not be accompanied by inhibition of spontaneous movement, so that the response to adrenaline may be purely motor[4] or biphasic (see Figure 2). In the three preparations tested the response to *l-nor*-adrenaline was purely motor. In the same concentration as *l*-adrenaline it produced a longer though less vigorous contraction of the gut. Acetylcholine also causes a contraction in this preparation, but is less potent in this respect than adrenaline. The effect of acetylcholine 10^{-7} is roughly equal to that of adrenaline 2×10^{-8}. Atropine sulphate in a concentration of 10^{-8} abolishes or prevents the effect of the acetylcholine while leaving the action of adrenaline but little modified. If, however, the concentration is raised 100 fold to 10^{-6} the motor effect of adrenaline 2×10^{-8} on the gut is prevented. This effect is shown in Figure 1.

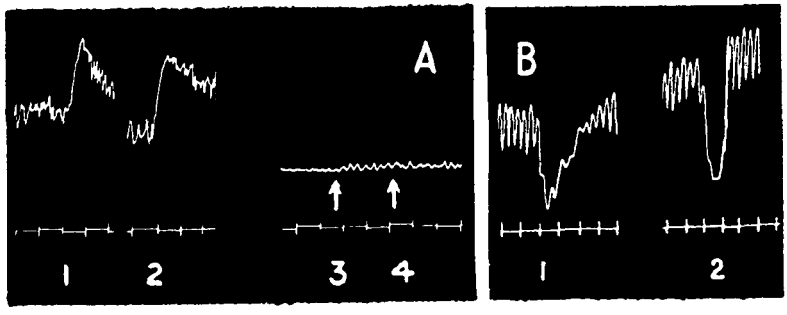


Fig. 1.—A. Isolated duodenum of duck. At 1. and 3. adrenaline 2×10^{-8}, at 2. and 4. acetylcholine 10^{-7}. Between 2. and 3. atropine sulphate 10^{-6}. B. Isolated rectal cæcum of fowl. At 1. and 2. adrenaline 10^{-9}. Between 1. and 2. atropine sulphate 2×10^{-6}. Time in 10 secs.

The motor effect of adrenaline 10^{-7} is not inhibited by *l*-hyoscine or *l*-hyoscyamine 10^{-5}, but is reduced to about one half by *l*-homatropine 10^{-5} and is abolished by atropine and trasentin 6H in the same concentration. The latter two compounds have a profoundly inhibitor effect on the tone and spontaneous movement of the gut in these high concentrations, the former have not. In the rectal cæcum of the fowl the spontaneous movements are not inhibited by atropine (2×10^{-6}) nor is the inhibitor action of adrenaline (10^{-8}) modified by atropine in this concentration (see Figure 1).

The antihistamine compounds have been shown to have varying potencies as spasmolytic agents against contraction of smooth muscle caused by acetylcholine, histamine and barium (Graham[6]), and in the course of that work some inhibition of the effect of adrenaline on the

blood pressure of the chloralosed cat was noted after addition of these compounds. Accordingly the effect of neoantergan (Dews and Graham[7]) was tested upon the action of adrenaline and *l-nor*-adrenaline on duck duodenum. As already stated the effect of adrenaline was to produce

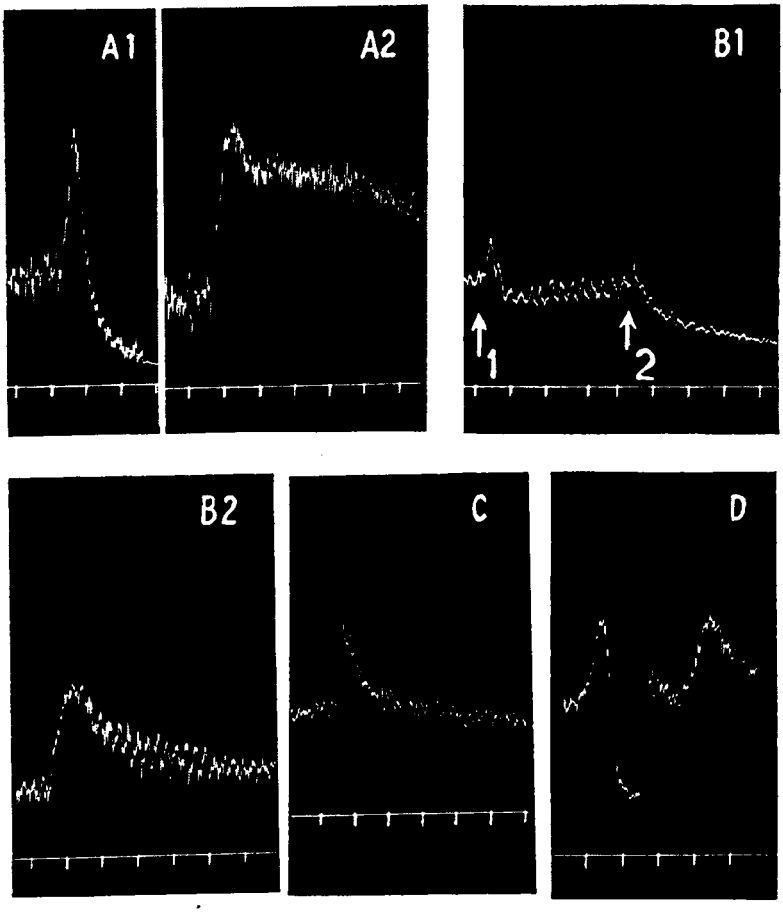


FIG. 2.—Isolated duodenum of duck. A1, biphasic response to *l*-adrenaline 10^{-7}. A2, motor response to *l-nor*-adrenaline 10^{-7}. B_1, neoantergan 10^{-6} injected at arrow 1, inhibits the motor part of the biphasic response to *l*-adrenaline 10^{-7} injected at arrow 2. B2, as in A2 but in the presence of neoantergan 10^{-6}. C, effect of *l*-adrenaline 10^{-7} 5 minutes after neoantergan 10^{-6} had been washed out twice. Motor part of biphasic response only restored. D, as in A1 and A2 after 10 minutes washing out of neoantergan 10^{-6}. Time in 10 secs.

a biphasic response, that of *l-nor*-adrenaline to produce a motor response. Neoantergan in a concentration of 10^{-6} abolished the motor part of the response to adrenaline and reduced the inhibitor part of the response; the response to *l-nor*-adrenaline was partially inhibited (see Figure 2B). A concentration of 10^{-5} of neoantergan abolished the re-

sponse to either compound. The characteristic effect of both compounds could be restored after repeated washings, but the motor part of the response to adrenaline returned before the inhibitor portion of the response (see Figure 2C). These reactions are illustrated in Figure 2.

The Effect of Neoantergan (2768 r.p.) on the Response to Adrenaline of Isolated Gut of Rabbit and Guinea-pig, and Isolated Pregnant Uterus of Rabbit

In view of the inhibition by neoantergan of the action of adrenaline on the duodenum of ducks a similar trial of its effect on the action of adrenaline on isolated rabbit duodenum and guinea-pig duodenum was made. As is well known, the movement of such specimens is regular and is inhibited by adrenaline (10^{-8}). Preparations vary in the degree and duration of inhibition caused by this concentration of adrenaline. Neoantergan in a concentration of 10^{-5} usually caused the gut to relax and inhibited spontaneous movements; during this inhibition adrenaline caused no relaxation, but as the inhibition was already maximal no further effect could be expected. After repeated washings of the preparation the spontaneous contractions were small and frequent and the tone was high (Fig. 3F and 3H). Under these conditions in some preparations the action of adrenaline (10^{-8}) was abolished or reversed (3F) for a variable time, but ultimately returned. In other preparations (3H) the action of adrenaline was not abolished. These variations were related to the amount of neoantergan administered to the bath, the duration of its action, and to the period allowed for recovery and the number of wash-outs given. Inhibition of adrenaline action was better seen with specimens of duodenum than of ileum.

Neoantergan in a concentration of 10^{-5} occasionally caused immediate spasm of the muscle preparation which lasted for some 2 minutes and was followed by increased spontaneous activity (3B). Under these circumstances the action of adrenaline could be tested in the presence of neoantergan (3B), when it was found to be less effective. After repeated washing the action of adrenaline was abolished (3C) and only partially restored after 10 washes at minute intervals (3D). Atropine, homatropine and trasentin had no effect on adrenaline inhibition of rabbit gut in low concentrations (10^{-8}); higher concentrations diminished gut movement so that further action by adrenaline could not be tested.

The isolated strip of pregnant uterus of rabbit responds to the addition of adrenaline (10^{-8}) with a transient spasm (Fig. 4A). Neoantergan in a concentration of 10^{-5} produces a prolonged increase in tone with frequent strong contractions superimposed upon it (4B). If adrenaline is added at the beginning or during the course of this period of increased tone caused by neoantergan the muscle is inhibited (4C and 4D). The action of adrenaline on this preparation is thus reversed by neoantergan in the concentration stated. Backman and Lundberg[8] have shown that atropine has a similar effect on rabbit uterus.

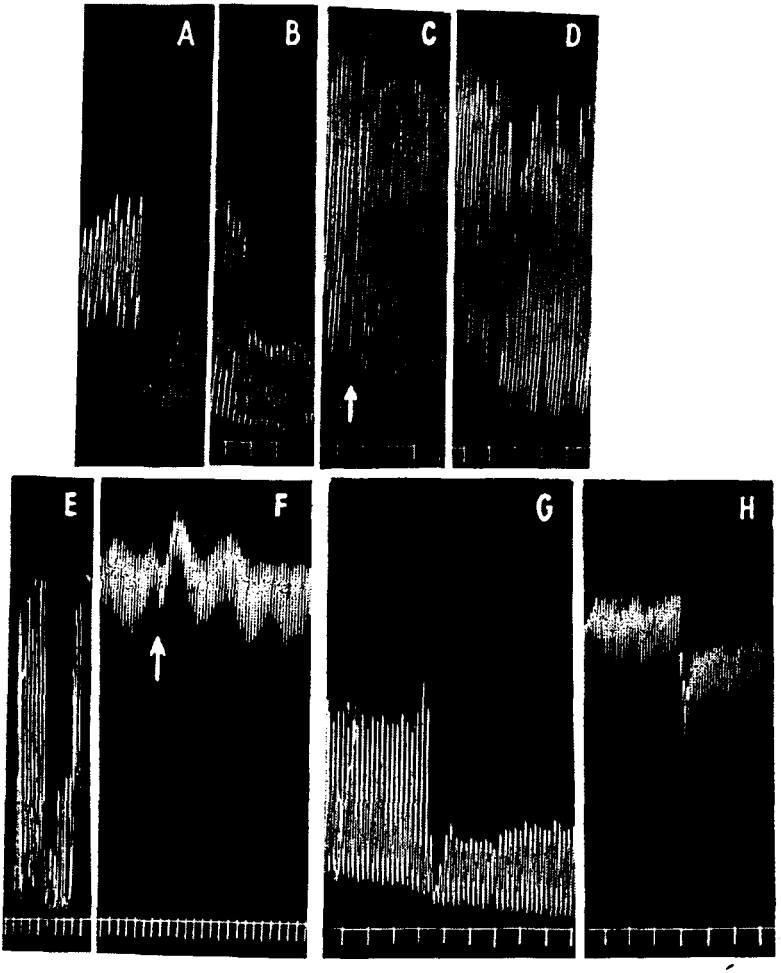


FIG. 3.—Isolated strips of rabbit duodenum. A. effect of adrenaline 10^{-8}. B. effect of A. in presence of neoantergan 10^{-5} C. effect of A. after 5 changes of bath fluid at 1 minute interval. D. as in C. after 10 minutes interval. E. effect of adrenaline 10^{-8} on a fresh strip of duodenum. F. reversal of adrenaline effect 5 minutes after washing out neoantergan 10^{-5}. G. effect of adrenaline 10^{-8} on a fresh strip of duodenum. H. failure to modify action of adrenaline 5 minutes after neoantergan 10^{-5}. Contrast with E. and F. Time in 10 secs.

THE EFFECT OF ATROPINE, HYOSCINE, TRASENTIN AND NEOANTERGAN ON THE ACTION OF ADRENALINE ON THE BLOOD PRESSURE OF SPINAL CATS

Bussel[2] illustrates the fall in blood pressure in etherised cats which results from injection of atropine, and the inhibition of the response of the blood pressure to stimulation of the thoracic sympathetic chain and of the contraction of the nictitating membrane after administration of adrenaline to spinal cats, following upon injection of atropine. Hyoscine did not have these actions.

In a series of spinal cats adrenaline and *l-nor*-adrenaline were injected intravenously and the similar effects recorded. A comparison of the pressor effects of the two compounds on spinal cats indicated that *l-nor*-adrenaline has 165 per cent. of the pressor activity of *l*-adrenaline (B.P.) which agrees closely with the assay carried out by Tainter *et al*[5] on dogs. It was found that injection of neoantergan 0·5 mg./kg. caused a transient small rise in blood pressure and slightly potentiated the

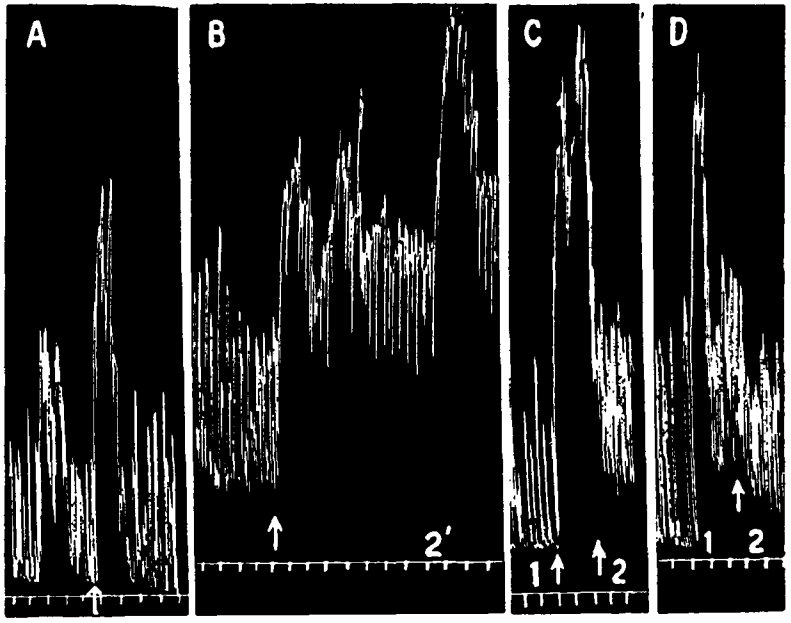


FIG. 4.—Isolated uterus of pregnant rabbit. A. motor effect of adrenaline 10^{-5}. B. prolonged motor effect of neoantergan 10^{-5}. C. adrenaline 10^{-4} added at arrow 2 inhibits the spasm caused by neoantergan 10^{-5} added at arrow 1. D. the effect of adrenaline 10^{-4} added at arrow 2 is reversed by previous addition of neoantergan 10^{-5} at point 1. Time in 2 minutes intervals.

action of these compounds (5B), while a dose of 4·0 mg./kg. caused a transient fall in blood pressure and inhibited the action of these compounds. This effect was brief. 8·0 mg./kg. caused a profound fall in blood pressure and further blocked the action of adrenaline and arterenol. Higher doses were too toxic to allow of further tests; benadryl and antistine[6] cause a sharp fall in the blood pressure of chloralosed cats into which they are injected intravenously in doses of 1·0 mg./kg. Similar action has been cited by Bussel[2] as evidence of anti-adrenaline activity by atropine sulphate.

Trasentin 6H in a dose of 1·0 mg./kg. slightly potentiates the pressor effect of adrenaline or *l*-arterenol, while 4·0 mg./kg. inhibits the response. Larger doses of trasentin 6H (8·0 mg./kg.) completely suppress the response to adrenaline and *l*-arterenol, but are toxic, and under such

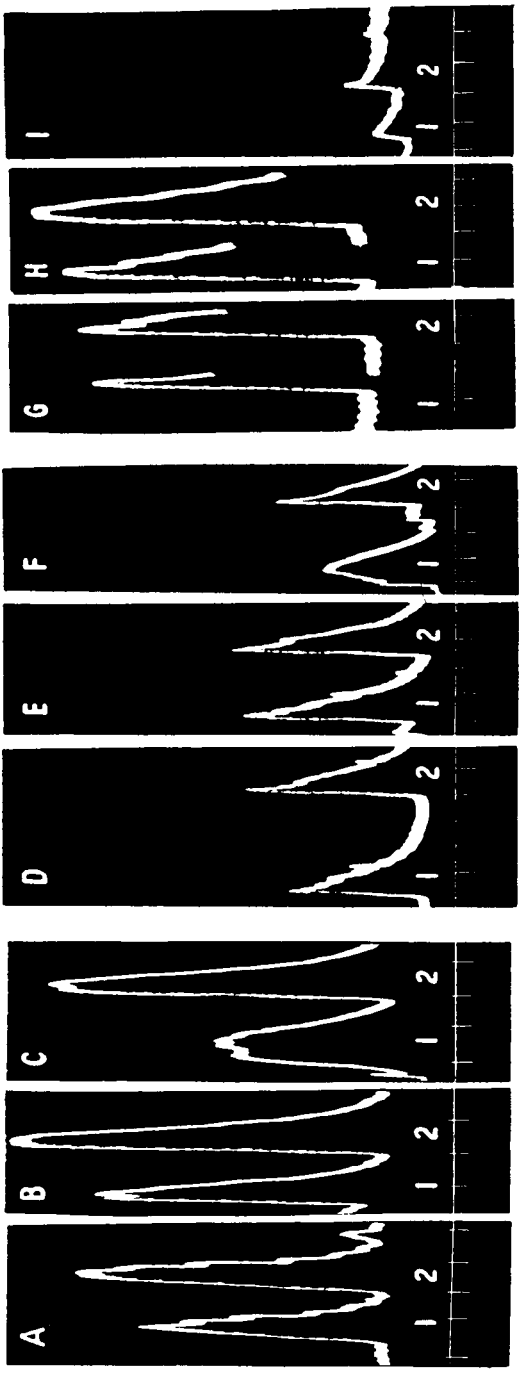


FIG. 5.—Carotid blood pressure taken from spinal cats of 2·5 to 3·5 kg. wt. Time in 30 sec. A1. pressor effect of *l-nor*-adrenaline 1·0 μg./kg. A2. pressor effect of *l*-adrenaline 2·0 μg./kg. B1. and 2. as above after neoantergan 0·5 mg./kg. showing potentiation of effect of A1. and A2. C1. effect as in A1. inhibited after neoantergan 4·0 mg./kg. C2. effect as in A2. still potentiated. Reversal of the order of administration of C1. and C2. reverses this picture which is due to the transient action of 4·0 mg./kg. of neoantergan in blocking adrenaline activity. D1. and D2. as in A1. and A2. but different cat. E1. and E2. as in D1. and D2. but after trasentin 1·0 mg./kg., effect potentiated. F1. and F2. as in D1. and D2. but after trasentin 4·0 mg./kg., effect inhibited. G1. and G2. *l-nor*-adrenaline 1·0 μg./kg. and *l*-adrenaline 1·6 μg./kg. respectively. H1. and H2. as in G1. and G2. but after atropine sulphate 1·0 mg./kg., effect potentiated. I1. and I2. as in G1. and G2. but after atropine sulphate 20 mg./kg., effect inhibited.

conditions the failing heart and low blood pressure are incapable of responding to any stimulus.

l-Hyoscine (1·0 mg./kg.) slightly potentiates the effect of adrenaline and *l-nor*-adrenaline, but has no further effect in doses up to 8·0 mg./kg. Atropine sulphate 1·0 mg./kg. slightly potentiates the effect of *l*-adrenaline and *l-nor*-adrenaline, 4·0 mg./kg. has a stronger action, and 20·0 mg./kg. almost abolishes the effect of both compounds without undue toxic actions on the cardio-vascular system of the cats (5I).

In drakes anæsthetised with pentobarbitone solution injected into the breast muscles followed by inhalation of ether, the administration of atropine sulphate 1·0 mg./kg. intravenously causes a sharp transient fall in blood pressure of about 20 mm. Hg. This is similar to the effect seen in etherised cats and rabbits. Doses of 10·0 mg./kg. of atropine do not inhibit the pressor effect of adrenaline 10 μg./kg. intravenously in this preparation.

Discussion

Much recent work has increased our knowledge of the close and complex relationships between the functions of the sympathetic and parasympathetic nervous systems and the actions of acetylcholine and adrenaline and other compounds which modify their activity. The effect of such a substance on any organ is closely related to the dose administered. McDowall[9] and Elio[10] have shown that small doses of acetylcholine may stimulate the heart; larger doses inhibit it. Acetylcholine may potentiate the effect of adrenaline on the heart and blood vessels, and the opposite may occur in the central nervous system (Burn[12]). Small doses of adrenaline lower the blood pressure, larger doses raise it. Many sympathomimetic compounds such as ephedrine, amphetamine, tyramine, cocaine and other local anæsthetics have a different effect on the activity of adrenaline according to the concentration in which they are administered (Jang[13], Graham and Gurd[14]).

Sherif[15] has shown that the hypogastric nerve to the uterus of the bitch is cholinergic in nature but is not paralysed by atropine, while Secker[16] has shown that salivary secretion in the dog following upon injection of adrenaline and sympathetic stimulation is inhibited by atropine. The inhibitory action of atropine on the effect of adrenaline on duck duodenum is moderately potent (atropine 10^{-6} is effective), while the inhibitory action of transentin 6H and homatropine is less effective (10^{-5}) and *l*-hyoscine and *l*-hyoscyamine appear to be relatively ineffective. There is no apparent relation between the activity of these compounds in inhibiting the action of adrenaline on this preparation and their relative potencies in inhibiting the action of acetylcholine (Graham and Gunn[17]). No action could be shown of these spasmolytic compounds in preventing the inhibitor effects of adrenaline in isolated rabbit gut since in effective doses they are themselves powerful inhibitors of spontaneous movement in this preparation. While atropine and trasentin 6H in doses of 1·0 mg./kg. produce a transient fall in blood pressure

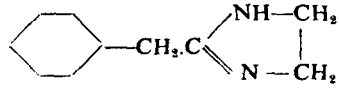
in cats which have a good vascular tone and a high blood pressure (ether or chloralose anæsthesia) it takes much greater quantities of these drugs to inhibit the pressor effect of adrenaline in the spinal cat. It may be therefore that the transient fall in blood pressure seen in etherised cats after atropine, trasentin, etc., 1·0 mg./kg. is a non-specific spasmolytic effect on the arteriolar muscle in high tone rather than a specific anti-adrenaline effect as Bussel[2] suggests, but the clear diminution in the pressor response to stimulation of the thoracic sympathetic chain in spinal cats shown by that author and the great inhibition of the pressor response to injected adrenaline and *nor*-adrenaline illustrated in Figure 5 of this report show that atropine exerts a blocking effect on the motor actions of adrenaline, as does trasentin 6H. Bussel[2] attributes this blocking effect of atropine to its structural resemblance to cocaine which when present in low concentration competes with adrenaline for receptors on the enzymes which inactivate adrenaline and thus potentiates the effect of the latter, and in high concentrations competes with adrenaline for cell receptors and thus inhibits its action (MacGregor[18]). In support of Bussel's explanation of atropine in high concentration inhibiting adrenaline activity is the finding that doses of 1·0 mg./kg. (1/20 of the adrenaline-inhibiting dose) increased the pressor response to adrenaline, as did *l*-hyoscine, trasentin 6H and neoantergan.

Neoantergan[6,7] inhibits spasm of smooth muscle caused by histamine, acetylcholine and barium. It also blocks the relaxation of some but not all smooth muscle caused by histamine. It appears from the experiments described that it not only inhibits the pressor effect of adrenaline on the vascular bed but reverses the motor effect on the uterus, and inhibits the relaxor effect of adrenaline on some preparations of gut. Its action in this respect differs from that of dibenamine[19] which can reverse the motor response of rabbit uterus and the pressor response of the cat to administration of adrenaline, but does not affect the inhibitory action of adrenaline on gut. The properties of adrenolysis and sympatholysis are shown in varying degree by ergot[20], yohimbine[21], 2-diethyl-aminoethyl-1 : 4-benzdioxan (833F) and 2-piperidinomethyl-1 : 4-benzdioxan (933F), ephedrine, cocaine, atropine, trasentin 6H, neoantergan, priscol[22], etc. Of these compounds only priscol has been stated to show the property described for neoantergan of blocking the inhibitory action of adrenaline on some segments of rabbit gut. Ahlquist *et al*[22] do not consider this a specific effect of priscol because of the irregularity of appearance of the phenomenon.

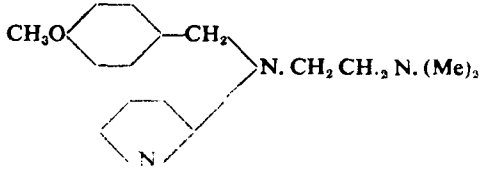
Adrenaline may be written as $R_1.CHOH.CH.NH.R_2$. A similar ethylamine chain may be visualised with varying degrees of ease in all the adrenolytic compounds mentioned.

In dibenamine it appears thus: —$Cl.CH_2.CH_2.N(CH_2.Ph)_2$.

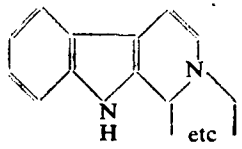
in priscol: —



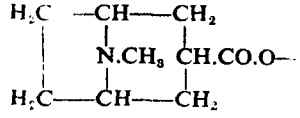
in neoantergan (2786 R.P.):—



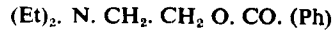
-in yohimbine[28]:—



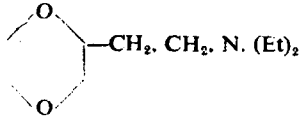
in atropine:—



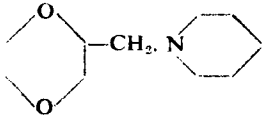
in trasentin 6H:—



in 833 F:—



and 933 F:—



The possession of a common element of structure would account for common properties on the basis of such a theory as that propounded by MacGregor[18] for the effect of cocaine on adrenaline action.

That the effect of spasmolytic compounds in blocking adrenaline action is essentially similar to their effect in blocking *l-nor*-adrenaline is of interest in view of the suggestion of Euler[24], Bacq and Fischer[25] and Gaddum and Goodwin[26] that *nor*-adrenaline is sympathin. West[27] compared *l*-adrenaline and *dl-nor*-adrenaline, and found that both had a powerful inhibitory effect on isolated segments of rabbit gut. The same effects were found above with *l-nor*-adrenaline but in duck gut, in which *l*-adrenaline produces a biphasic response, *l-nor*-adrenaline is purely motor.

SUMMARY

1. Isolated segments of duodenum from the duck react to the addition of adrenaline by a biphasic response, contracting and relaxing. Atropine, trasentin 6H, homatropine and neoantergan (2786 R.P.)

abolish this contractile response. Hyoscine and hyoscyamine have no effect in concentrations of 10^{-5}.

2. Rabbit gut is inhibited by adrenaline and *l-nor*-adrenaline. This action is abolished after neoantergan has been in contact with some segments of gut.

3. Rabbit uterus contracts with adrenaline or neoantergan. In the presence of the latter the former inhibits movement of the uterus.

3. The pressor response to adrenaline and *l-nor*-adrenaline in spinal cats is inhibited by atropine, trasentin and neoantergan in high doses (atropine sulphate 20·0 mg./kg.).

4. Certain differences between *l*-adrenaline and *l-nor*-adrenaline are discussed.

5. A tentative hypothesis is offered to account for the adrenolytic activity found in a wide variety of compounds. This is an extension of MacGregor's well-known explanation for the reactions of cocaine and adrenaline, based on certain common structural features in the adrenolytic agents discussed.

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