

THE ANTAGONISM OF COCAINE TO THE ACTION OF CHOLINE 2,6-XYLYL ETHER BROMIDE AT SYMPATHETIC NERVE ENDINGS

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Choline 2,6-xylyl ether bromide (TM10) prevents contraction of the nictitating membrane and of the spleen in response to sympathetic nerve stimulation. It was confirmed that this effect was due to prevention of the release of noradrenaline ("sympathin") at the nerve endings. The intravenous injection of cocaine before choline xylyl ether blocked the action of the latter drug. However, when atropine was injected before the cocaine the effects of large doses of choline xylyl ether were not completely blocked. After block had been produced by choline xylyl ether the injection of cocaine partially restored both the secretion at the sympathetic nerve endings and the responses of the organs supplied by the nerves. Phenoxybenzamine did not restore the secretion at the nerve endings after it had been inhibited by choline xylyl ether, indicating that the latter did not cause an increased uptake of sympathin by the receptors.

While investigating the pharmacological effects of nuclear substituted choline phenyl ethers, Hey and Willey (1954) noticed that choline 2,6-xylyl ether bromide (TM10; hereafter called choline xylyl ether) blocked the responses of the nictitating membrane of the cat to pre- and post-ganglionic stimulation of the cervical sympathetic nerve. They also showed that choline xylyl ether had a local anaesthetic action almost as powerful as that of cocaine. This led them to suggest that choline xylyl ether might act by suppressing conduction in postganglionic sympathetic nerve fibres. Exley (1957) extended the observations of Hey and Willey (1954) by showing that choline xylyl ether produced a transient ganglionic block, but that it did not prevent the passage of impulses along the nerve fibres. He also showed that the impulses arriving at the nerve endings were in some way rendered ineffective, as noradrenaline did not appear in splenic venous blood when the splenic nerves were stimulated after doses of choline xylyl ether.

While working on the effects of diphtheria toxin on the responses of smooth muscle to various sympathomimetic amines, it was necessary to prevent the secretion of noradrenaline (sympathetic transmitter, "sympathin") at the ends of the sympathetic nerve fibres. Although choline xylyl ether appeared to be ideally suited for this purpose it was surprising to find that the drug was without

effect. Our experimental conditions differed from those of previous workers only in that the animal had been given cocaine. Further experiments showed that cocaine was extremely effective in antagonizing the action of choline xylyl ether, and the results of these experiments are reported here.

METHODS

Operative Procedures.—The experiments were performed on cats anaesthetized with ether followed by chloralose (80 mg./kg. body weight). In all experiments systemic arterial pressure was measured with a mercury manometer. The preganglionic fibres of the left cervical sympathetic nerve were separated from the vagus nerve and prepared for stimulation. In some animals the separation of the two nerves was continued above the superior cervical ganglion so that postganglionic stimulation was possible. In a few cats the right sympathetic nerve was also isolated. The contraction of the nictitating membrane in response to nerve stimulation was recorded on smoked paper with an isotonic frontal-writing lever.

When changes in splenic volume were to be recorded, the abdomen was opened in the midline and the spleen was exposed. The vessels running between the spleen and the stomach were cut between ligatures. In this way the spleen was mobilized and, after the splenic nerves had been isolated, it was enclosed in an oncometer. Changes of volume were recorded with a piston volume recorder.

The amount of noradrenaline liberated in response to splenic nerve stimulation was determined by the technique of Brown and Gillespie (1957). The abdomen was opened and the spleen mobilized as described above. The inferior and superior mesenteric arteries were ligated and the intestines were removed from the lower part of the colon to the upper part of the duodenum. A polyethylene cannula, treated with silicone to inhibit clotting, was inserted into the superior mesenteric vein pointing towards the liver, and a loop of thread passed round the portal vein a few millimetres nearer to the liver than to the junction of the splenic and mesenteric veins. The splenic nerve was isolated, divided near the coeliac ganglion and prepared for stimulation. In most experiments the coeliac ganglion was excised, and where this was not done the splanchnic and hepatic nerves were divided. The adrenal glands were removed in four cats, but this procedure did not materially affect the concentration of noradrenaline in the plasma. Apart from the samples collected, blood loss during the experiments was slight, but in some instances the arterial pressure fell appreciably during the operation. In these cats 15 to 25 ml. of warm saline was slowly injected intravenously for 30 to 40 min. so that the experiments could be continued under reasonably stable conditions.

Plasma Samples.—As stimulation of the splenic nerve was begun tension was put on the loop around the portal vein and blood was collected for the 10 sec. of stimulation and for the following 20 sec. Sufficient heparin was included in each tube to prevent clotting. Blood in excess of 6 ml. was returned to the animal to reduce exsanguination. The retained blood was cooled and the plasma separated. The plasma was refrigerated until the following day, when its noradrenaline content was estimated using the blood pressure of a pithed rat which had received atropine. The haematocrit value of each sample of blood was measured in order that the output of noradrenaline/min. might be calculated.

Nerve Stimulation.—The cervical sympathetic nerve was laid across the electrodes in a pool of liquid paraffin. It was stimulated for 1 sec. in every minute by a Palmer Student Stimulator at a rate of 30 pulses/sec. of 4 V. and duration of 0.35 msec. In experiments in which samples of blood were collected, the splenic nerve was given similar stimuli, but the timing was 10 sec. in every 10 min. When changes in the splenic volume were being recorded the nerve was stimulated for periods of 2 sec. and the stimulus strength was adjusted to give responses commensurate with the capacity of the recording apparatus.

Solutions.—All drugs were dissolved in isotonic saline. Doses were given into a cannula in the femoral vein.

RESULTS

Experiments on the Nictitating Membrane of the Cat

Actions of Choline Xylyl Ether.—In six experiments choline xylyl ether (10 mg./kg. body weight) produced a biphasic effect on the contractions of the nictitating membrane in response to

nerve stimulation. *Phase one* was an immediate reduction in the size of the contraction lasting for about 8 min. In some experiments, the contractions entirely disappeared during the first 2 to 3 min. after injection and then reappeared and increased until they were nearly the same height as they were before the drug was given. In other experiments, the contractions were reduced to about one-third of their initial height. *Phase one* was reduced or absent when the amount of choline xylyl ether used was decreased to 5 mg./kg. *Phase two* consisted of a gradual diminution in the size of the contractions of the nictitating membrane until after varying intervals they disappeared altogether (Fig. 1). This phase usually began after 8 min. or more, but in one of the cats with the larger dose of choline xylyl ether it appeared to follow *phase one* immediately, and no contraction was recorded 2 min. after injection of the drug. When preganglionic stimulation had failed to elicit a response, stimulation of the postganglionic fibres was without effect. With doses of 10 mg./kg. of choline xylyl ether the average time for complete disappearance of contractions was 16 min. (range, 2 to 30 min., $n=5$). With doses of 5 mg./kg. *phase two* was prolonged and the average time for disappearance of contractions was 69 min. (range, 20 to 107 min.).

It was considered that frequent stimulation of the nerve might hasten the disappearance of contractions, and in two cats this hypothesis was tested. Both cervical sympathetic nerves were prepared for stimulation, and the right nerve was stimulated for 1 sec. in every minute for 1 hr. Stimulation of the left nerve was begun and 10 mg./kg. choline xylyl ether injected immediately afterwards. In both cats there was a slightly faster fall in the height of the contractions on the right side, but the difference between the two sides was small. Choline xylyl ether therefore appeared to exert the same effect whether or not the nerve was stimulated before the drug was given.

The Effects of Atropine on Sympathetic Nerve Block Produced by Choline Xylyl Ether.—In early experiments all animals received atropine (2 mg./kg., intravenously) to prevent any response of the nictitating membrane to stimulation of cholinergic fibres (Bacq and Fredericq, 1935), and to prevent any muscarine actions of choline xylyl ether itself. This rather large dose of atropine caused a diminution of the size of contraction of the nictitating membrane in eleven out of twelve cats.

Experiments were performed to determine whether or not atropine influenced the time taken by choline xylyl ether to prevent completely the

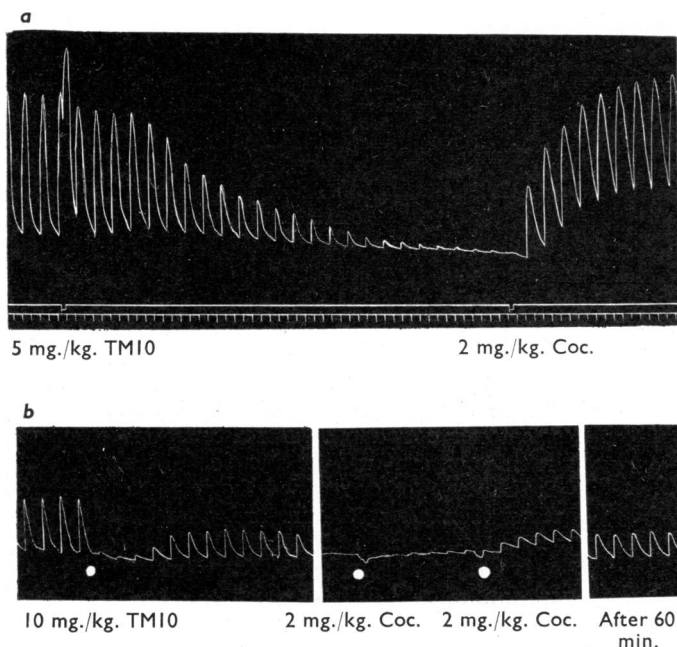


FIG. 1.—The effect of choline xylyl ether (TM10) on the responses of the nictitating membrane to supramaximal stimulation of the cervical sympathetic nerves in a cat anaesthetized with chloralose. *a*, Shows reversal of the block produced by 5 mg./kg. of choline xylyl ether by 2 mg./kg. of cocaine. *b*, Shows cocaine to be less effective in reversing the action of 10 mg./kg. of choline xylyl ether. The first injection of cocaine in *b* was given 30 min. after choline xylyl ether; the last section of the tracing was obtained 60 min. after injecting cocaine. Time, 30 sec.

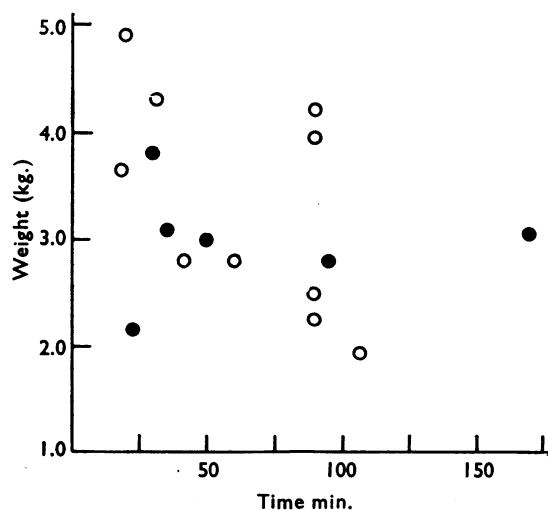


FIG. 2.—The variation in the time taken by a dose of 5 mg./kg. of choline xylyl ether to eliminate the responses of the nictitating membrane to stimulation of the cervical sympathetic nerve. ●, in the presence of 2 mg./kg. of atropine. ○, in the absence of atropine.

response of the nictitating membrane to nerve stimulation. It also seemed possible that the time course of the effect of choline xylyl ether was not necessarily directly related to the dose, calculated on a body weight basis. In these experiments, the time taken by 5 mg./kg. of choline xylyl ether to block the responses of the nictitating membrane to stimulation of the cervical sympathetic nerve was compared between cats given 2 mg./kg. of atropine and cats not given atropine. The results of these experiments are plotted in Fig. 2 so that they indicate both the influence of atropine and of the dose (on a body weight basis) on the action of choline xylyl ether. Although the results could be interpreted to mean that atropine accelerated the action of the choline xylyl ether, any such effect was small and the routine administration of atropine was discontinued.

The Action of Cocaine.—There was antagonism between the effects of cocaine and choline xylyl ether on *phase two* but not on *phase one* of the action of the latter drug on the nictitating membrane.

When 2 mg./kg. of cocaine was given intravenously there was an immediate increase in the size of the contraction of the nictitating membrane followed by a rise in the base line due to prolongation of the effects of stimulating the cervical sympathetic nerve. In one experiment in which 5 mg./kg. of choline xylyl ether was given after cocaine, there was no change in the size of the contraction over the ensuing 100 min. In three experiments, 10 mg./kg. of choline xylyl ether was injected after cocaine. The size of the contraction was immediately reduced. This corresponded to *phase one* of the effects of large doses of choline xylyl ether already referred to, and no difference was detected between this *phase one* reaction and that occurring in the absence of cocaine. In two of the experiments after recovery from *phase one* due to choline xylyl ether, there was no reduction in the height of the contractions. In one experiment the recovery from *phase one* was not complete, and when further doses of choline xylyl ether were given the contractions were diminished and it was inferred that antagonism between the drug and cocaine was not absolute.

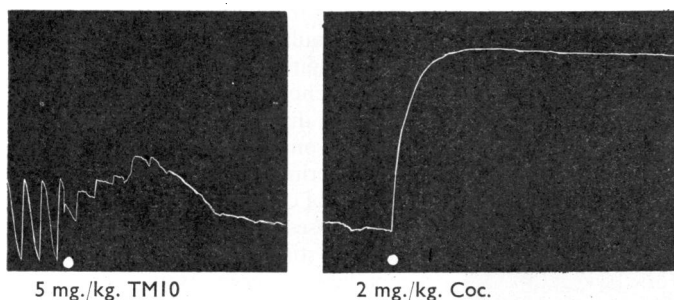


FIG. 3.—Contraction of the nictitating membrane in response to supramaximal stimuli applied to the cervical sympathetic nerve for 1 sec. every minute. Choline xylyl ether blocked the responses in 17 min.; the injection of cocaine then produced a contracture which was maintained only so long as stimulation at regular intervals was continued.

After the membrane response had been abolished by 5 mg./kg. of choline xylyl ether, the administration of cocaine led to an immediate return of the contraction to its former height, or almost so, within 10 min. After larger amounts of choline xylyl ether, cocaine caused recovery to only a third or a quarter of the former height of contraction. Further injections of cocaine tended to increase the height of the contraction, but in no experiment did it increase to more than half of the original height. These effects are illustrated in Fig. 1.

The antagonism between cocaine and choline xylyl ether appeared to be dependent upon the quantities of the two drugs present. Three cats were given 5 mg./kg. of choline xylyl ether and when the membrane response had disappeared 2 mg./kg. of cocaine was given. In each case the response was restored, but a further dose of 10 mg./kg. of choline xylyl ether abolished the response in two cats in 113 and 78 min. respectively, whilst in the third animal the response was only slightly diminished after 131 min. The third cat appeared to be less sensitive to choline xylyl ether since the times taken for inhibition of the membrane response after the first doses of choline xylyl ether were 23, 45, and 100 min. in the three cats respectively. It was also noted in two other experiments in which 10 mg./kg. of choline xylyl ether had been given previously, that although it had been impossible to restore the membrane response to its original value by a single injection of cocaine, three successive injections of 2 mg./kg. of cocaine each progressively increased the strength of the contractions.

In three of the fifteen experiments in which cocaine was injected after choline xylyl ether it produced a contracture of the nictitating membrane and not a restoration of the normal responses. This is illustrated in Fig. 3. The contracture was dependent upon regular nerve stimulation, for it slowly declined when stimulation was discontinued, and again became apparent when stimulation was restarted.

Experiments on the Spleen

The Effect of Choline Xylyl Ether on the Splenic Response to Nerve Stimulation.—Stimulation of the splenic nerve produced a decrease in splenic volume. In three experiments the injection of 5 mg./kg. of choline xylyl ether reduced the response to nerve stimulation after 10 min. and after 20 min. the response was practically abolished. The effects of a dose of 10 mg./kg. of the drug were similar and are illustrated in Fig. 4. This inhibition in about 20 min. occurred whether or not the stimulation was regular during that time. This confirmed the observations on the nictitating membrane that regular stimulation of the nerve fibre before the

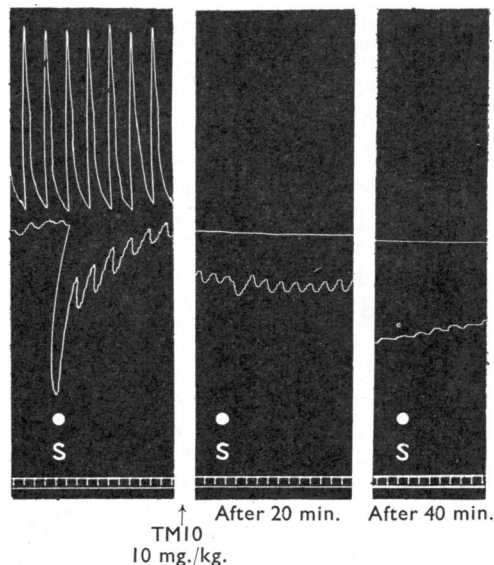


FIG. 4.—Upper record: contractions of the nictitating membrane in response to supramaximal stimulation of the cervical sympathetic nerve (for 1 sec. in every 60 sec.). Lower record: contractions of the spleen in response to stimulation of the splenic nerves (S) for 2 sec. in every 10 min. The injection of 10 mg./kg. of choline xylyl ether (TM10) completely abolished the responses of the nictitating membrane in 20 min. At this time the splenic response was slight and it disappeared completely after 40 min. Time, 30 sec.

injection of choline xylyl ether did not hasten the effects of the drug.

The injection of 10 mg./kg. of choline xylyl ether after the animal had been given 2 mg./kg. of cocaine produced only a small reduction in the response of the spleen to stimulation of the splenic nerves 43 min. later. This is illustrated in Fig. 5. The animal from which this record was taken was not atropinized and the injection of choline xylyl ether produced a transient muscarine effect.

The Effect of Choline Xylyl Ether on the Secretion of Noradrenaline at Splenic Nerve Endings.—The experiments were divided into two groups. In both groups atropine was given at the beginning of the experiment and samples of blood were taken both in the resting state and during nerve stimulation. In the first group of experiments cocaine was injected before choline xylyl ether and in the second

group the order was reversed. The results are given in Table I. The resting and stimulation samples for the control periods of both groups of experiments have been combined. It is apparent that when choline xylyl ether was injected before cocaine it greatly reduced the secretion of noradrenaline, and the amount of noradrenaline recovered from the blood was the same in the second stimulation sample taken 30 min. after the administration of choline xylyl ether as in the resting sample taken only 10 min. after the drug. Cocaine, given before choline xylyl ether, raised the amount of noradrenaline liberated by nerve stimulation. However, there were insufficient experiments to be certain that this increase in noradrenaline was significant. The injection of cocaine before choline xylyl ether prevented the latter drug from reducing noradrenaline liberation to the low levels recorded in its absence, but there was some reduction of the secretion. When cocaine was administered after choline xylyl ether had blocked the effects of nerve stimulation there was some recovery of the secretion of noradrenaline. The antagonism between choline xylyl ether and cocaine seen on the nictitating membrane response was therefore also seen on the spleen.

The estimates for the noradrenaline in the resting samples in Table I are high. A few experiments were performed on animals from which the adrenal glands had been removed in an attempt to determine whether or not the glands were the source of this high background. There was no significant reduction of the resting noradrenaline in these experiments, and it was concluded that the high background was a consequence of the repetitive stimulation of the cervical sympathetic nerve, which was a constant feature of these experiments.

In one experiment phenoxybenzamine was given to prevent the adsorption of noradrenaline on the receptors. The order in which drugs were given was: choline xylyl ether, 5 mg./kg.; phenoxybenzamine, 10 mg./kg.; and cocaine, 2 mg./kg. After the choline xylyl ether there was no increase in the noradrenaline secreted when the splenic nerves were stimulated. The injection of phenoxybenzamine did not restore secretion which was, however, restored when cocaine was injected. All blood samples were in duplicate.

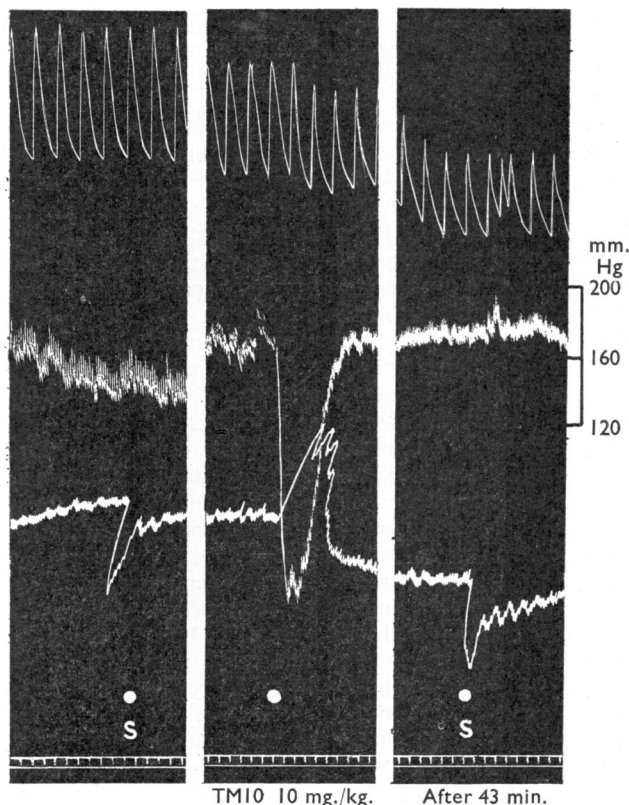


FIG. 5.—Cat given 2 mg./kg. of cocaine intravenously. Upper record: contractions of the nictitating membrane in response to supramaximal stimulation of the cervical sympathetic nerve for 1 sec. in every minute. Middle record: blood pressure. Lower record: contraction of the spleen in response to stimulation of the splenic nerves (S). The injection of choline xylyl ether (TM10) produced transient muscarine effects. The contractions of the nictitating membrane and the spleen were not blocked 43 min. after the injection of choline xylyl ether. Time, 30 sec.

TABLE I

THE INFLUENCE OF CHOLINE XYLYL ETHER AND COCAINE ON THE RELEASE OF NORADRENALINE AT SPLENIC NERVE ENDINGS

Splenic venous blood was collected for noradrenaline assay during resting periods and after stimulation of the splenic nerve. The numerals are estimates of the amount of noradrenaline liberated into splenic venous blood during a period of 1 min. There was a 10 min. interval between the injection of a drug and collection of the first (resting) sample, and also between the collection of stimulation samples.

	Noradrenaline Release (ng./min.)				
	Com- bined Controls	Choline Xylyl Ether Alone	Cocaine Alone	Choline Xylyl Ether after Cocaine	Cocaine after Choline Xylyl Ether
Resting ..	223	68	213	173	95
First stimulation ..	391	121	562	358	165
Second ..	443	70	528	360	147
No. of cats ..	9	5	4	4	5

DISCUSSION

The observation of Hey and Willey (1954) that choline xylyl ether blocks the response of the nictitating membrane to stimulation of the cervical sympathetic nerve has been confirmed. It has also been shown that with doses of 10 mg./kg. of choline xylyl ether the effect on the responses of the nictitating membrane was biphasic in the sense that there was an initial transient inhibition before the drug caused complete block. It seems probable that the transient inhibition of the membrane responses is due to two factors, both of which were demonstrated by Exley (1957). The first of these is a transient ganglion-blocking action; the second is a temporary depression of the responses of the membrane to adrenaline. Hey and Willey (1954) demonstrated also that choline xylyl ether temporarily inhibits the rise in blood pressure which normally follows an injection of adrenaline.

There was a great variation in the time taken in different animals for choline xylyl ether to block completely the nictitating membrane response. Bain and Fielden (1957) showed that the drug inhibits the synthesis of noradrenaline from dopamine, and it might be supposed, therefore, that the time taken by choline xylyl ether to block the effects of stimulating a nerve fibre would be proportional to the time taken to deplete the store of noradrenaline in the fibre. In our experiments repeated stimulation of the nerve for 1 sec. in every minute for 1 hr. before the injection of choline xylyl ether did not materially affect the time taken to produce block. Had regular stimulation of the nerve before the injection of the drug reduced the time taken to produce block, it would have indicated that the variations were due to

differences in the amount of stored noradrenaline. It is possible that in our experiments the rate of synthesis or replacement of the noradrenaline in the nerve fibre was at least equal to the rate of its liberation.

The action of atropine in these experiments is more difficult to assess. In the absence of atropine an injection of cocaine prevented choline xylyl ether, given subsequently, from exerting its effect. In the presence of atropine, however, cocaine delayed but did not abolish the effect of choline xylyl ether. It is possible that atropine and cocaine, having somewhat similar molecules, competed for the same receptors.

Bacq and Fredericq (1935) have shown that, when physostigmine was instilled into the conjunctival sac of the eye of a cat, the responses of the nictitating membrane to stimulation of the cervical sympathetic nerve were increased. Furthermore, the responses were inhibited by intravenous doses of atropine, which latter observation we have confirmed in the present work. It was therefore suggested that there might be some cholinergic innervation of the nictitating membrane. However, since Exley (1957) showed that choline xylyl ether blocked the effect of sympathetic but not of vagal stimulation on the cat heart, the observation that this drug will completely block the nictitating membrane response to nerve stimulation in the absence of atropine casts some serious doubt upon the possibility of there being any cholinergic fibres present. The diminution in contraction of the nictitating membrane after atropine might be better correlated with the observation of Thompson (1958) that the responses of the isolated nictitating membrane to doses of adrenaline are reduced by atropine, which seems to imply that the action of atropine in this respect is directly on the muscle.

In confirmation of the results of Exley (1957) we have shown that choline xylyl ether prevents the secretion of noradrenaline at splenic nerve endings. The previous injection of cocaine antagonized this effect. After choline xylyl ether had prevented the secretion of sympathin, cocaine partially restored it. The antagonistic action of cocaine was reflected in the pattern of events observed when the cervical sympathetic nerve was stimulated and the contractions of the nictitating membrane were recorded.

The disappearance of noradrenaline from the plasma in the splenic vein after a dose of choline xylyl ether cannot be explained by postulating a bigger uptake of noradrenaline by the receptors: when the receptors were blocked with phenoxybenzamine (Brown and Gillespie, 1957; Brown, Davies, and Gillespie, 1958) the effect was merely

to increase the resting concentration of noradrenaline in the plasma and not to restore the response to nerve stimulation.

Brown and Hey (1956) found that various nuclear-substituted choline phenyl ethers, including choline xylyl ether, inhibited the enzymatic destruction of adrenaline by guinea-pig liver. It is therefore highly unlikely that choline xylyl ether works by promoting rapid enzymatic destruction of noradrenaline, though if this were so the action of cocaine would be easy to explain, as it has been shown to antagonize the action of amine oxidase *in vitro* (Philpot, 1940).

There is some evidence that choline xylyl ether interferes with the synthesis of noradrenaline. Coupland and Exley (1957) showed that the injection of choline xylyl ether into rats depleted the adrenal glands of their stores of adrenaline and noradrenaline. Furthermore, Bain and Fielden (1957) reported that choline xylyl ether inhibited the synthesis of noradrenaline from dopamine by homogenates of human chromaffin-cell tumours. Earlier Bain and Fielden (1956) had demonstrated that dopamine antagonized the blocking action of choline xylyl ether and that the latter did not inhibit the synthesis of dopamine itself. It has, therefore, to be explained why added dopamine did not antagonize the action of choline xylyl ether in the homogenate. One possible interpretation of the

facts, so far as they are known at present, is that choline xylyl ether can enter into combination with dopamine and with cocaine. The effect of the combination with dopamine would be to prevent the formation of noradrenaline unless an excess of dopamine is provided. Combination of choline xylyl ether with cocaine would effectively prevent the former from combining with dopamine.

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