Brit. J. Pharmacol. (1961), **17**, 539–551.

EFFECT OF MORPHINE ON SOME SYMPATHETICALLY INNERVATED EFFECTORS

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(*Received October* 3, 1961)

Morphine, in doses of 0.5 to 55 mg/kg, inhibited contraction of the nictitating membrane of the cat following stimulation of the postganglionic sympathetic nerve fibres. Morphine was more effective at low than at high frequencies of stimulation, independently of the size of contraction of the membrane; the speed of contraction was reduced at all frequencies. Cocaine potentiated the contraction of the nictitating membrane following nerve stimulation more at low than at high frequencies, and antagonized the action of morphine. These findings, and the absence of an effect of morphine on the action of injected noradrenaline, make it likely that morphine interferes with the release of noradrenaline from the postganglionic nerve endings in the nictitating membrane. Morphine had no effect on the cardioaccelerator action of the cardiac nerves and inconsistent results were obtained on the emptying of the spleen after stimulation of the splenic nerves.

In recent years evidence has accumulated which shows that morphine inhibits the responses of certain autonomically innervated effectors. Thus in the isolated guinea-pig ileum it depresses the peristaltic reflex elicited by distending the lumen (Schaumann, 1955; Kosterlitz & Robinson, 1955, 1957), and in the rat and the rabbit it reduces the cardiac slowing produced by stimulating the right vagus nerve (Kosterlitz & Taylor, 1959a). Further, morphine inhibits the contraction of the nictitating membrane resulting from stimulation of the cervical sympathetic (Trendelenburg, 1957); it also diminishes the relaxing effect of sympathetic stimulation on the longitudinal muscle of the guinea-pig jejunum (Szerb, 1961).

Little is known about the mechanism of these actions of morphine, except that in guinea-pig ileum the release of acetylcholine is depressed after distension of the lumen (Schaumann, 1956) or coaxial electrical stimulation (Paton, 1956, 1957). In this paper an attempt has been made to analyse the action of morphine more thoroughly by studying its effect on the responses of the nictitating membrane, the sinoauricular node and the smooth muscle of the spleen to stimulation of their postganglionic sympathetic fibres. Some of the results have already been reported briefly elsewhere (Kosterlitz & Taylor, 1959b; Cairnie, Kosterlitz & Taylor, 1961).

METHODS

Adult cats of either sex were used. In most experiments the cats were anaesthetized by intraperitoneal injection (5 ml./kg) of a solution of 10% (w/v) urethane and 1% (w/v) chloralose, but when the cardiac nerves were stimulated, spinal preparations made by the

method of Kosterlitz, Krayer & Matallana (1955) were used. Since morphine depresses the respiratory centre, all experiments were carried out under artificial respiration.

In the experiments on the nictitating membrane, the left superior cervical ganglion and postganglionic trunk were exposed by the removal of the pharynx, larynx, upper part of the oesophagus and the long deep neck muscles. The postganglionic trunk was dissected out, secured by a fine ligature and severed from the ganglion, which was removed.

In the experiments on the heart rate, the approach to the cardiac nerves was through the first intercostal space. They were secured by a fine ligature just distal to the stellate ganglion which was then excised. Atropine (1 mg/kg) was injected intravenously to block the effect of stimulation of any aberrant vagal fibres.

In the experiments on the spleen, the abdomen was opened in the mid-line and the intestines were removed from the mid-duodenum to the terminal colon. The vascular connexions between the spleen and the stomach and omentum were tied and cut and the omentum removed. The splanchnic nerves were cut on both sides to prevent stimulation of the adrenal medullae. The splenic nerves were dissected away from the splenic artery, ligatured and cut.

For stimulation, the nerves were placed on perspex-embedded platinum electrodes and the area of dissection flooded with liquid paraffin. The nerves were stimulated with biphasic rectangular pulses from a Grass stimulator, using an RF-coupled output stage to isolate the stimuli from earth. Unless otherwise stated, the stimuli were supramaximal, and were applied for 45 sec every 3 to 4 min to the cervical postganglionic trunk, for 1 min every 4 to 5 min to the cardiac nerves and for 90 or 105 sec every 5 to 10 min to the splenic nerves.

The contractions of the nictitating membrane were recorded isotonically with a frontal writing lever and the change in the volume of the spleen by means of a plethysomograph chamber and piston recorder. The resting heart rate was determined from the electrocardiogram for a period of 10 sec at the beginning of each successive minute; when the cardiac nerves were stimulated, the heart beats were counted from the 15th to the 25th and the 45th to the 55th sec of the period of stimulation.

When stimulus frequency/response curves were determined, the contraction of the nictitating membrane or the decrease in volume of the spleen was plotted as a percentage of the maximal response against the logarithm of the stimulus frequency. Before the administration of morphine two control curves were obtained. Then morphine was injected and the series of observations was repeated. In the experiments on the heart rate, stimulus frequency/response curves were not constructed. In any one such experiment, we used the same frequency throughout, so chosen as to give an increase in heart rate of about 50% of the possible maximum.

The blood pressure in the left femoral artery was measured by means of a condenser manometer. All injections of drugs were made into the right femoral vein.

The doses of morphine hydrochloride, nalorphine hydrochloride, bretylium tosylate and dibenyline (phenoxybenzamine) hydrochloride refer to the salts, those of noradrenaline to the base.

RESULTS

Contraction of the nictitating membrane

Stimulus frequency/response curves. These curves, which were obtained by applying supramaximal stimuli at different frequencies to the postganglionic nerve fibres, were sigmoid in shape. In most preparations the response was just noticeable at a frequency of 0.125 stimuli/sec, and the maximum response was obtained at a frequency of 16 or 24 stimuli/sec (Fig. 1). Any one curve was reproducible within narrow limits for periods of 1 to 2 hr.

At the lowest frequencies (0.125 to 0.25 stimuli/sec) the fusion of the contractions was incomplete, and in particularly sensitive membranes, stimulated at 0.0625 stimuli/sec, a discrete contraction was observed in response to each stimulus.

Effect of morphine on the stimulus frequency/response curve. In all the 15 experiments in which morphine was given after control stimulus frequency/response curves had been established, small doses reduced the contractions of the nictitating membrane. This effect was much greater at low than at high frequencies (Fig. 1). The onset of inhibition was very rapid, occurring within 1 min after injection.

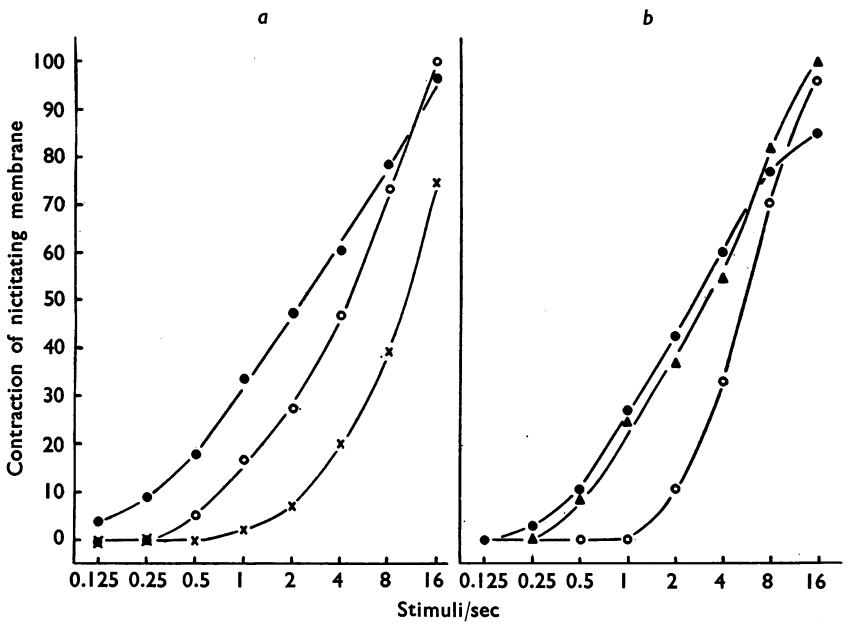

Fig. 1. Stimulus frequency/response curves showing the effects of morphine alone and of nalorphine given subsequently on the contractions of the nictitating membrane of the cat when the post-ganglionic nerve fibres were stimulated supramaximally. Abscissa: frequency of stimulation. Ordinate: contraction of the nictitating membrane as a percentage of the maximal contraction. a: ●——●, control; ○——○, after 1 mg/kg morphine; X——X, after additional 10 mg/kg morphine. b: ●——●, control; ○——○, after 15 mg/kg morphine; ▲——▲, after subsequent injection of 7.5 mg/kg nalorphine.

There was a considerable variation in the degree of inhibition found in different cats given the same dose of morphine, and, in any one cat, large doses were relatively less effective than small doses. For example, after doses of morphine of 0.5 to 2 mg/kg body weight, the response to 2 stimuli/sec was reduced by an average of 55 to 60%, the variation being from 33 to 76% in all experiments. However, there was a decrease of only 70% (range 68 to 73% in 3 experiments) after doses as large as 40 to 55 mg/kg body weight.

The morphine antagonist, nalorphine, reversed the effect of morphine either partly or, in some experiments, completely (Fig. 1b).

Correlation of the magnitude of the morphine effect with either the size of the contraction or the frequency of stimulation. The greater effectiveness of morphine in reducing the size of contractions produced by stimulation at low frequencies could be associated with either the smaller size of the response under these conditions (Trendelenburg, 1957) or with the frequency of stimulation. These alternative possibilities were tested by constructing stimulus frequency/response curves for supramaximal and submaximal stimuli and examining the effect of morphine on them.

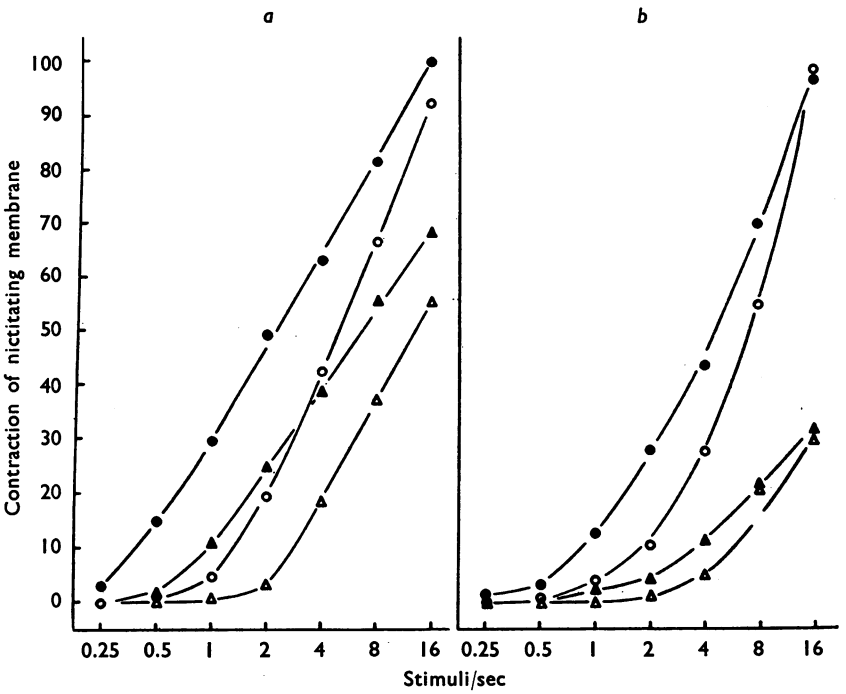

Fig. 2. Stimulus frequency/response curves showing the effects of morphine on the contractions of the nictitating membrane of the cat when the postganglionic nerve fibres were stimulated supramaximally and submaximally. The strength of the submaximal stimulus was chosen to give at 16 stimuli/sec a contraction of about 70% (a) and 30% (b) of the supramaximal stimulus. Abscissa: frequency of stimulation. Ordinate: contraction of the nictitating membrane as a percentage of the maximal contraction. ●——●, control, supramaximal stimulation; ▲——▲, control, submaximal stimulation; ○——○, after 2 mg/kg morphine, supramaximal stimulation; △——△, after 2 mg/kg morphine, submaximal stimulation.

The submaximal stimuli used were such as to give, at a frequency of 16 stimuli/sec, responses of either 70 or 30% of those obtained at this same frequency with the supramaximal stimuli. The effect of reducing the strength of the stimulus was to diminish the responses at high as well as at low frequencies (Fig. 2).

The effect of morphine on the responses obtained with submaximal, as with supramaximal, stimulation was greater at low than at high frequencies (Fig. 2). Or, to put it differently, when the size of the control response at 16 stimuli/sec was.

as a result of submaximal stimulation, only 30% of that obtained with supramaximal stimulation at this frequency, morphine had no inhibitory effect (Fig. 2b). On the other hand, a contraction of this same size produced by supramaximal stimulation at 2 stimuli/sec was reduced to one-third by the same dose of morphine. Therefore the effect of morphine was not correlated with the size of the contraction but with the frequency of stimulation.

Effect of morphine on the contractions of the nictitating membrane due to injected noradrenaline. Trendelenburg (1957) showed that morphine did not reduce the small contractions caused by injection of small doses of noradrenaline. It was, however, of importance to make quite sure that morphine had no inhibitory effect

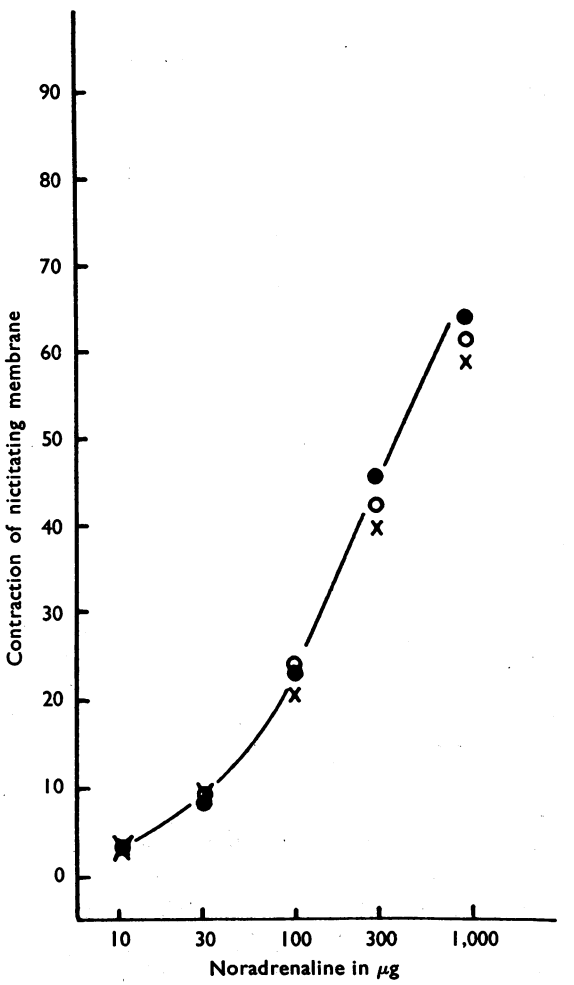

Fig. 3. Dose/response curves showing the effect of morphine on the contraction of the nictitating membrane of the cat resulting from the intravenous injection of noradrenaline. Abscissa: dose of noradrenaline. Ordinate: contraction of the nictitating membrane as a percentage of the maximal contraction obtained by electrical stimulation of the postganglionic fibres. ●——●, control; o——o, after 2 mg/kg morphine; X——X, after additional 3 mg/kg morphine.

on contractions caused by larger doses of noradrenaline. By injecting into a cat of 2.2 kg doses of noradrenaline varying between 0.01 and 1 mg, a dose/response curve was constructed which included sizes of contractions of between 5 and 60% of the size of the maximal contraction obtainable by stimulation of the nerve (Fig. 3). Morphine in doses up to 5 mg/kg had no effect on the responses of the membrane to doses of between 0.01 and 0.1 mg noradrenaline, whereas those to 0.3 and 1 mg were reduced by only 13 and 18% respectively.

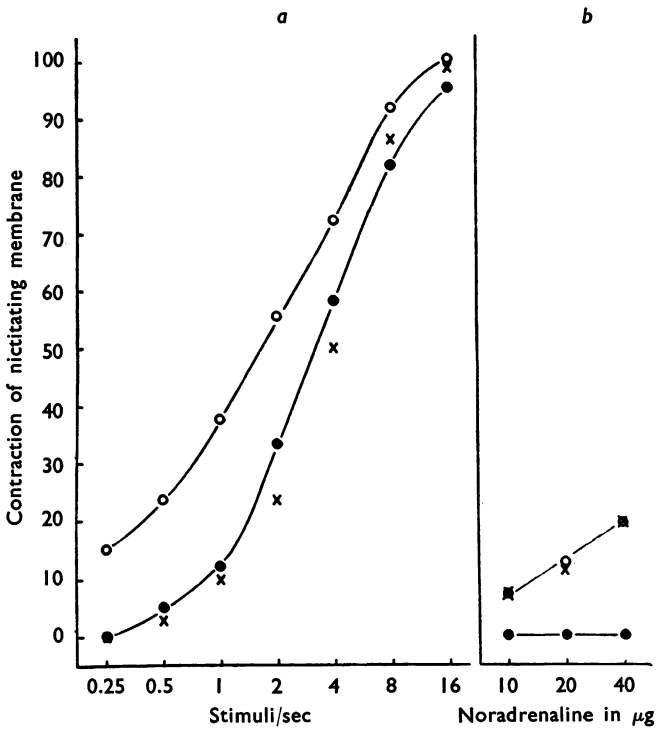

Fig. 4. The effect of cocaine followed by morphine on the contractions of the nictitating membrane of the cat produced by (a) supramaximal stimulation of the postganglionic nerve fibres and (b) intravenous injection of noradrenaline. Abscissa: (a) frequency of stimulation; (b) dose of noradrenaline. Ordinate: contraction of the nictitating membrane as a percentage of the maximal contraction. ●——●, control; ○——○, after 2 mg/kg cocaine; X——X, after subsequent injection of 1 mg/kg morphine.

Relationship between the effects of morphine and cocaine on the stimulus frequency/response curve. Cocaine, which is known to increase the sensitivity of the nictitating membrane to injected noradrenaline, was more effective in potentiating the responses to stimulation at low than at high frequencies (Fig. 4a). When morphine was given after sensitization of the membrane by cocaine, it caused the stimulus frequency/response curve to return to the level of the control curve, but it did not affect the responses to injected noradrenaline (Fig. 4a and b). When, on the other hand, morphine was given first, cocaine in suitable doses almost restored the stimulus frequency/response curve to normal (Fig. 5). The potentiating effect of cocaine, greater at low frequencies of stimulation, was not correlated with the

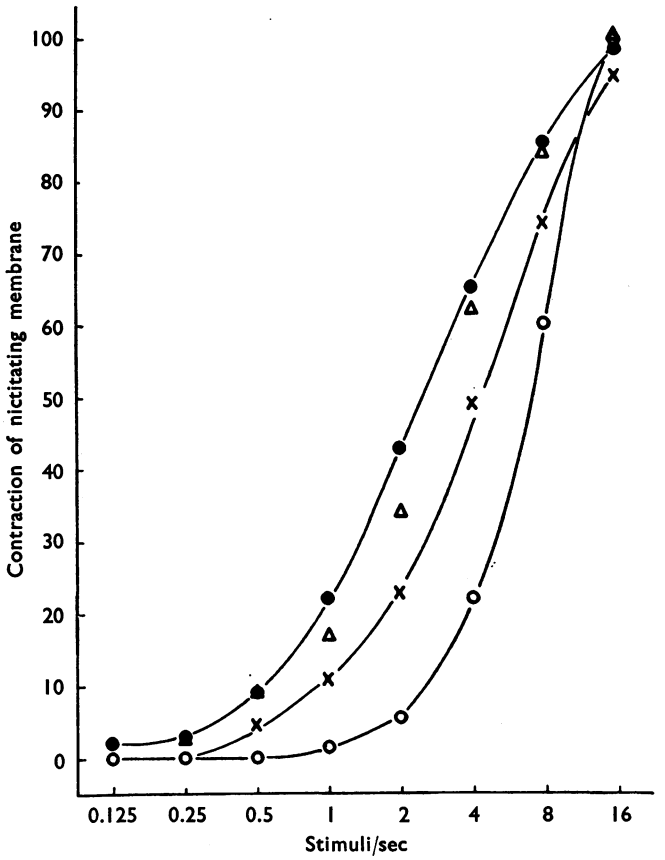

Fig. 5. The effect of morphine followed by cocaine on the contractions of the nictitating membrane of the cat produced by supramaximal stimulation of the postganglionic nerve fibres. Abscissa: frequency of stimulation. Ordinate: contraction of the nictitating membrane as a percentage of the maximal contraction. ●—● , control; ○—○, after 2 mg/kg morphine; X—X, after subsequent injection of 0.4 mg/kg cocaine; △—△, after additional 0.4 mg/kg cocaine.

size of the contraction. This was shown by the finding that sensitization of the membrane by increasingly large doses of cocaine produced proportionate and parallel shifts to the left of the noradrenaline dose/response curve, at least until the contractions were from 40 to 50% of the size of those caused by maximal electrical stimulation (Fig. 6).

When the postganglionic nerve fibres to a nictitating membrane, sensitized by administration of cocaine, were stimulated at a very low frequency, about 4 stimuli/min, a discrete contraction was observed for each stimulus. Morphine abolished this response completely (Fig. 7).

The effect of morphine on the rate of contraction of the nictitating membrane. Morphine increased the latent period and reduced the speed of contraction of the nictitating membrane due to postganglionic stimulation at low frequencies (Fig. 8a and b). This was not solely due to the decrease in the size of the contraction,

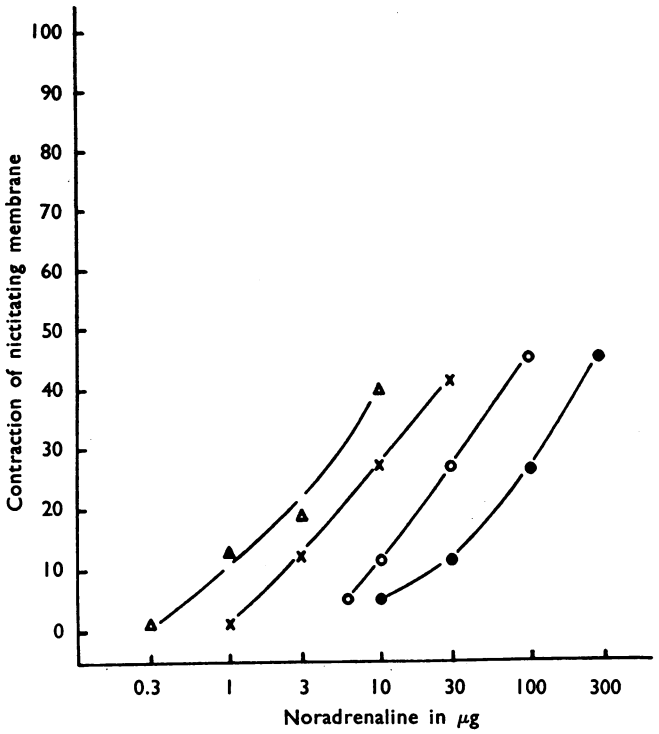

Fig. 6. The effect of cocaine on the responses of the nictitating membrane of the cat to intravenous injection of noradrenaline. Abscissa: dose of noradrenaline. Ordinate: contraction of the nictitating membrane as a percentage of the maximal contraction obtained by electrical stimulation of the postganglionic fibres. ● —— ●, control; ○ —— ○, after 0.3 mg/kg cocaine; X——X, after additional 0.6 mg/kg cocaine; Δ —— Δ, after additional 1.8 mg/kg cocaine.

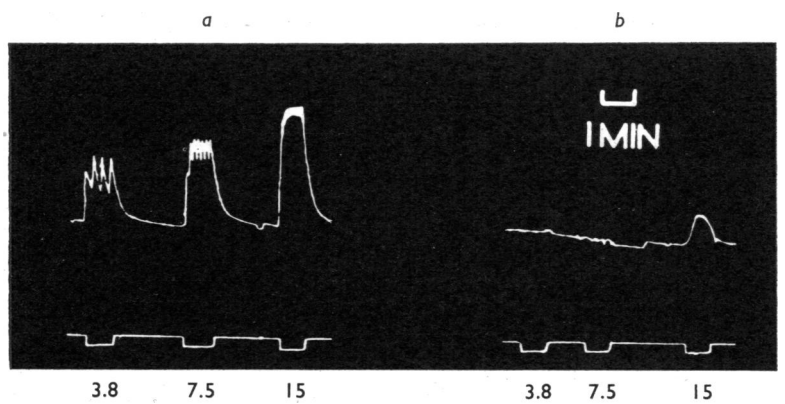

Fig. 7. The effect of morphine on the responses of the nictitating membrane of the cat to supramaximal stimulation at very low frequencies of the postganglionic nerve fibres. The membrane was sensitized by 2 mg/kg cocaine. (a) control; (b) after 2 mg/kg morphine. Numbers indicate stimuli/min.

since a similar reduction in size brought about by submaximal stimulation had much less effect on the speed of contraction (Fig. 8c). Although at high frequencies morphine reduced the height of contraction only very little, it was rather more effective in diminishing the speed of contraction (Fig. 8d and e).

Effects of bretylium and dibenyline on the frequency of stimulus/response curve. In view of the striking effect of morphine on the stimulus frequency/response curve, it was of interest to compare the effect of morphine with that of other drugs which reduce the responses of the nictitating membrane to nerve stimulation.

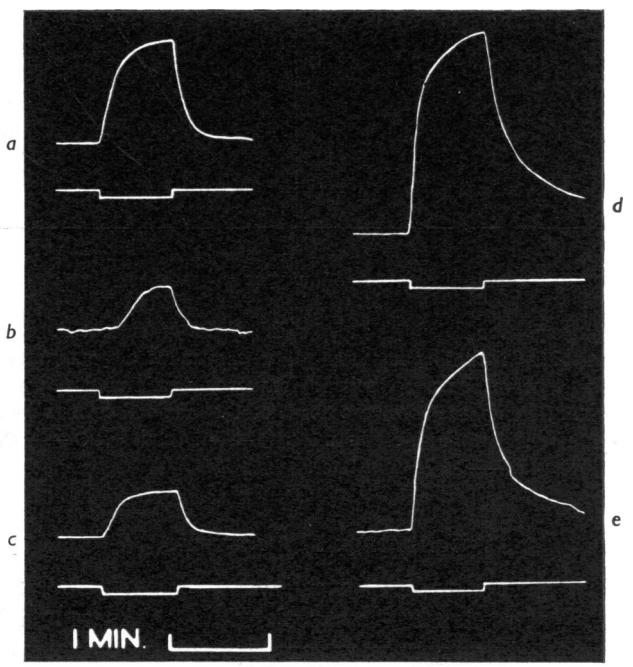

Fig. 8. The effect of morphine on the rate of contraction of the nictitating membrane of the cat following stimulation of the postganglionic nerve fibres. (a) control, 1 stimulus/sec, supramaximal; (b) after 2 mg/kg morphine, 1 stimulus/sec, supramaximal; (c) control, 4 stimuli/sec, submaximal; (d) control, 10 stimuli/sec, supramaximal; (e) after 2 mg/kg morphine, 10 stimuli/sec, supramaximal.

Bretylium, which, like morphine, does not depress the action of injected noradrenaline on the nictitating membrane (Boura & Green, 1959), depressed the contractions more at high than at low frequencies ; indeed, in doses up to 1.4 mg/kg, it had no effect on the contractions produced by stimulation at frequencies between 0.125 and 0.5 stimuli/sec (Fig. 9a).

Dibenyline, which antagonizes the action of noradrenaline on the nictitating membrane, reduced the responses at high as well as at low frequencies of stimulation (Fig. 9b).

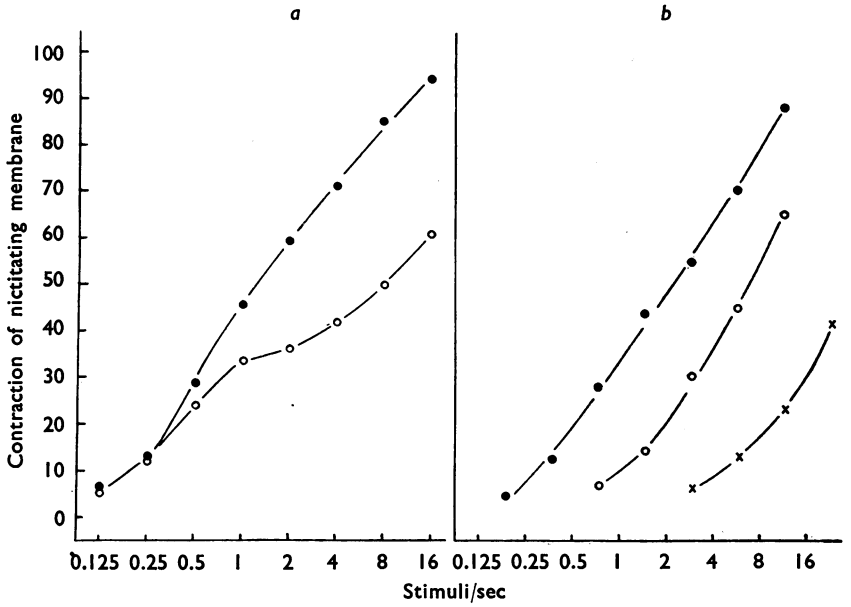

Fig. 9. Stimulus frequency/response curves showing the effects of (a) bretylium and (b) dibenyline on the contractions of the nictitating membrane of the cat produced by supramaximal stimulation of the postganglionic nerve fibres. Abscissa: frequency of stimulation. Ordinate: contraction of the nictitating membrane as a percentage of the maximal contraction. (a) ●——● control; ○——○, after 1.4 mg/kg bretylium. (b) ●——●, control; ○——○, 43–58 min after 0.3 mg/kg dibenyline; X——X, 30 min later.

Contraction of the spleen

It was impossible, in the course of 7 experiments, to demonstrate a constant effect of morphine on the contraction of the capsule of the spleen caused by stimulation of the splenic nerves. For instance, in one experiment, morphine inhibited the responses to electrical stimulation but the contractions caused by injection of noradrenaline were also reduced. In another experiment, after injection of morphine, there was a slight increase in the size of the responses to both stimulation of the splenic nerves and injection of noradrenaline. The morphine antagonist, nalorphine, depressed both types of response in one experiment, but in the other the results were equivocal.

Thus, although in the experiments on the nictitating membrane morphine clearly depressed the response to nerve stimulation but had no effect on the responses to injected noradrenaline, no such distinction could be shown to hold for the spleen.

Acceleration of the heart beat

In 8 experiments on the increase in heart rate brought about by the stimulation of the right cardiac nerves, no inhibitory effect of morphine was found (Fig. 10).

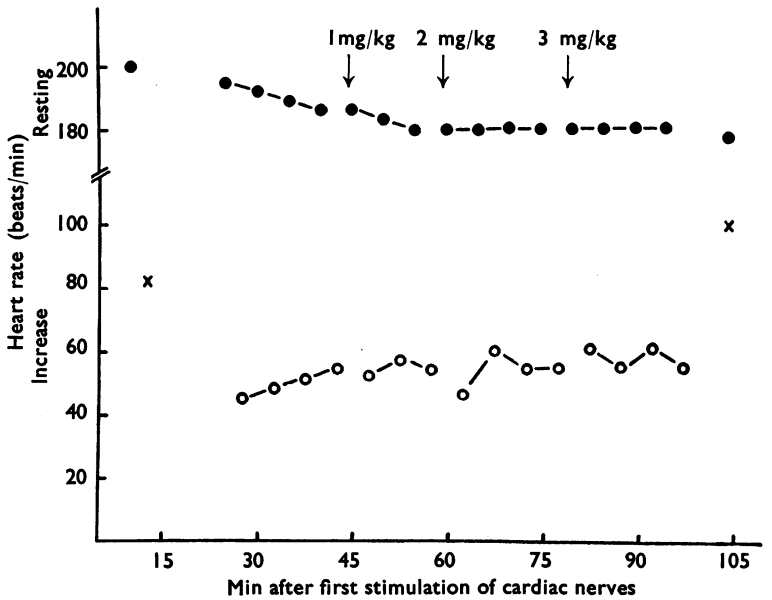

Fig. 10. The effects of morphine on the resting heart rate and on the increase in heart rate of the cat resulting from supramaximal stimulation of the cardiac nerves. Abscissa: time after first stimulation of the cardiac nerves. Ordinate: top, resting heart rate; bottom, increase in heart rate on stimulation. ● —— ●, resting heart rate; ○ —— ○, increase in heart rate after stimulation at 1.4 stimuli/sec; X, increase in heart rate after stimulation at 15 stimuli/sec (maximal effect). At the arrows, the indicated amounts of morphine were injected intravenously.

DISCUSSION

We have examined the effects of morphine on the responses of three different sympathetic effectors to postganglionic stimulation. There was inhibition of the contraction of the nictitating membrane, but there was no effect on the cardio-accelerator action of the cardiac nerves. It was doubtful if there was any action on the emptying of the spleen after stimulation of the splenic nerves. This inconsistency in the effect of morphine is in keeping with what has already been pointed out for certain parasympathetic effectors: morphine has an inhibitory effect on the vagal slowing of the heart in the rabbit but not in the guinea-pig, while the peristaltic reflex is suppressed in the guinea-pig but not in the rabbit (Kosterlitz & Taylor, 1959a).

There are a number of possible ways in which morphine might exert its inhibitory action. We have not yet investigated the possibility of an effect of morphine on impulse propagation in the postganglionic fibres innervating the nictitating membrane. Morphine, however, causes no changes in the conduction velocity in the A-B and C groups of fibres of the rabbit vagus nerve and the α, δ and C fibres of the cat saphenous nerve. Also, there is no delay in the recovery of excitability after a single conditioning stimulus in α and δ fibres of the cat saphenous nerve (Cairnie & Kosterlitz, 1961). Since the effect of morphine on the responses of the

nictitating membrane is greatest at low frequencies of stimulation, there would have to be a profound change in impulse propagation to account for our observations on the effect of morphine on the shape of the stimulus frequency/response curve. Indeed, only a more or less complete block of conduction could explain the suppression by morphine of the discrete responses to single stimuli seen in the membranes sensitized by cocaine. Further, it has been shown that when, by applying submaximal stimuli, a smaller number of nerve fibres is activated, the effect on the stimulus frequency/response curve is very different from the change observed after administration of morphine.

These findings, and the absence of an effect of morphine on the action of injected noradrenaline, seem to make it likely that morphine interferes with the release of noradrenaline from the postganglionic nerve endings in the nictitating membrane, a suggestion already made by Trendelenburg (1957). This is in agreement with the results obtained by Thompson (1960) on the isolated nerve-muscle preparation of the nictitating membrane; he found that morphine depressed the responses to supramaximal nerve stimulation but did not affect the contractions produced by adding adrenaline or noradrenaline to the bath fluid. So far, however, there is no direct evidence for such a diminution of noradrenaline release. The only site where morphine has been shown to reduce the amount of transmitter released is the cholinergically innervated smooth muscle of the guinea-pig ileum (Paton, 1956, 1957; Schaumann, 1956).

An important feature of the action of morphine is the fact that the reduction in the size of the contractions is greatest at low frequencies. A similar observation has already been made about the effect of morphine on the response of the sinoauricular node to stimulation of the right vagus nerve in the rabbit (Kosterlitz & Taylor, 1959a). Morphine differs in this respect from drugs which antagonize the action of the transmitter on the autonomic effector organ. The effect of such antagonists is always at least as great at high frequencies as at low frequencies. This is illustrated by the action of dibenyline on adrenergic neurones in the present series of experiments, and by that of atropine on the cholinergic neurones of the vagus (Kosterlitz & Taylor, unpublished observations).

Another observation which may be characteristic of the action of morphine on autonomic effectors is the decrease in the speed of contraction of the nictitating membrane, which is found not only at low but also, less obviously, at high frequencies of stimulation. It may be then that morphine exerts an inhibitory effect at these high frequencies although it does not reduce the size of contraction finally reached after 45 sec of stimulation. This may be in line with the findings of Kosterlitz & Taylor (1959a) that, although at high frequencies of stimulation morphine did not affect the degree of cardiac slowing resulting from stimulation of the vagus, there was a marked delay in the onset of the slowing.

There is a discrepancy between the conclusions drawn by Trendelenburg (1957) and ourselves about whether the effect of morphine is correlated with size of contraction, as he thought, or with frequency of stimulation. This probably arises from differences in experimental procedure. Trendelenburg stimulated for only 5 sec while we stimulated for 45 sec in order to be sure that at any given strength

and frequency of stimulation the maximum contraction was in fact obtained. A period of 5 sec is insufficient for this purpose, especially after morphine has been given, in view of its depressing effect on the speed of contraction.

Until the release of transmitter has been measured directly, we cannot know whether morphine reduces the amount liberated per volley to the same extent at low as at high frequencies of stimulation. Any attempt to explain more completely the effect of morphine on the stimulus frequency/response curve must remain speculative.

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