THE ACTION OF MORPHINE ON THE SUPERIOR CERVICAL GANGLION AND ON THE NICTITATING MEMBRANE OF THE CAT

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The intravenous injection of 0.05 to 2.5 mg. of morphine reduced the response of the nictitating membrane in the cat to pre- and post-ganglionic stimulation. This inhibitory action of morphine was due neither to inhibition of ganglionic transmission nor to a depressant action on the smooth muscle of the nictitating membrane. It is suggested that morphine inhibits the release of the sympathetic transmitter from the postganglionic nerve endings.

Small amounts of morphine (5 to 20 μg.) injected intravenously reduced or abolished the contraction of the nictitating membrane due to the injection of histamine, pilocarpine and 5-HT into the arterial blood supply of the superior cervical ganglion. This inhibitory action of morphine was due to an action on the ganglion cells, since such small amounts of morphine did not reduce the response of the nictitating membrane to postganglionic stimulation. Similar amounts of morphine did not abolish the stimulation of the ganglion by nicotine, tetramethyl-ammonium and potassium chloride.

The results provide further evidence for the view that histamine, pilocarpine, and 5-hydroxy-tryptamine have no " nicotine-like " properties but act on receptors of the ganglion cells different from the acetylcholine receptors.

Rocha e Silva, Valle and Picarelli (1953) were the first to observe that the stimulation of the isolated guinea-pig ileum by 5-hydroxytryptamine (5-HT) was due to an action of 5-HT on some nervous mechanism and not to a direct action on the longitudinal muscle. Further evidence for this view was provided by observations by Robertson (1953) and by Gaddum and Hameed (1954). The latter concluded that 5-HT acted on specific tryptamine receptors of the intestinal ganglion cells. Kosterlitz and Robinson (1955) found that small amounts of morphine abolished the response of the isolated guinea-pig ileum to 5-HT.

As it had been observed that 5-HT stimulated the superior cervical ganglion of the cat (Robertson, 1953 ; Trendelenburg, 1956a), it was of interest to investigate the effect of morphine on this nervous action of 5-HT. Histamine and pilocarpine have ganglionic actions very similar to those of 5-HT (Trendelenburg, 1954, 1955, 1956b) ; they were therefore included in this study.

METHODS

Cats of 2 to 5 kg. of both sexes were used. After inducing anaesthesia with ether, 80 mg./kg. chloralose was injected intravenously. Intra-arterial injections " to the ganglion " were made through the central end of the lingual artery during occlusion of the external carotid artery. The injected substance was thus diverted to the superior cervical ganglion. Injections " to the nictitating membrane " were made similarly, but without occlusion of the external carotid artery ; the injected substance then reached the nictitating membrane (Trendelenburg, 1954).

For postganglionic stimulation, the sympathetic trunk was exposed by removing the larynx, part of the oesophagus, and the *M. longus colli*. A unipolar electrode was hooked round the postganglionic sympathetic fibres at a distance of 2.4 mm. from the superior cervical ganglion. For preganglionic stimulation the cervical sympathetic chain was exposed, cut, and its peripheral end was placed on shielded electrodes. The nerve was then covered with warm liquid paraffin. In all experiments, the connexions of the superior cervical ganglion to the centres were divided by cutting the preganglionic fibres.

The movements of the nictitating membrane were recorded by attaching it to an isotonic lever fitted with a frontal writing point. Intravenous injections were made through the femoral vein.

For intra-arterial injections, the solutions were neutralized. The following substances were used:

morphine sulphate, histamine dihydrochloride, 5-hydroxytryptamine creatinine sulphate, pilocarpine nitrate, nicotine hydrogen tartrate, and tetramethylammonium bromide ; all weights refer to the salts.

RESULTS

Response of the Nictitating Membrane to Preganglionic Stimulation.—Morphine reduced the response of the nictitating membrane to submaximal preganglionic stimulation of the cervical sympathetic nerve. This was observed in eight preparations when intravenous injections of 20 μg., 100 μg., and 500 μg. morphine were given at 60 min. intervals. After 20 μg. the diminution was 18%, after 100 μg. it was 30% and after 500 μg. it was 52%. In these experiments preganglionic stimulation was applied every 30 sec. for 5 sec. The rate of stimulation was 2/sec. or 15/sec. The duration of the inhibitory action of morphine could not be determined accurately, as the response of the nictitating membrane to submaximal preganglionic stimulation usually failed to remain constant for periods of more than 10 to 15 min. In a few experiments, however, full recovery of the response was observed about 20 min. after the intravenous injection of 20 μg. morphine.

Response of the Nictitating Membrane to Postganglionic Stimulation.—Hebb and Konzett (1949) showed that morphine did not block transmission through the perfused superior cervical ganglion of the cat, but some ganglionic effects, such as those of histamine, are not readily seen in the perfused preparation. Experiments were carried out in which electrical stimulation was applied alternately to the pre- and post-ganglionic fibres of the superior cervical ganglion. Fig. 1 shows that the response of the nictitating membrane to both types of stimulation was reduced to the same extent after the intravenous injection of 100 μg. morphine, so that the effect was not due to ganglion block. When electrical stimulation was applied to the peripheral end of the postganglionic fibres after cutting them, intravenous injections of morphine caused a reduction of the response of the nictitating membrane. The presence of the ganglion cells was thus not essential for this effect of morphine.

Fig. 1 also shows that the response of the nictitating membrane to supramaximal stimulation of either the pre- or the post-ganglionic fibres was scarcely affected by the injection of 100 μg. morphine (compare Fig. 1a and c).

Influence of the Height of Contraction of the Nictitating Membrane.—Morphine depressed the response of the nictitating membrane to pre- or post-ganglionic stimulation to a greater extent when the stimulation rate was 2/sec. than when it was 15/sec. Supramaximal stimulation applied for 5 sec. at a rate of 2/sec. caused much smaller contractions of the nictitating membrane than supramaximal stimulation applied for the same period at the faster rate of 15/sec. In some experiments supramaximal stimulation (2/sec.) was applied alternately with submaximal stimulation (15/sec.) to the pre- or post-ganglionic fibres. The strength of submaximal stimulation was chosen so as to produce contractions of the nictitating membrane of similar height to those produced by supramaximal stimulation at 2/sec. Intravenous injections of 0.2 to 2.5 mg. morphine then reduced the response of the nictitating membrane to both types of stimulation to the same degree. Thus the response of the nictitating membrane to either pre- or post-ganglionic stimulation was the more reduced, the smaller the initial response of the nictitating membrane ; the rate of stimulation influenced the magnitude of the depressing effect of morphine only in so far as it determined the height of the contraction of the nictitating membrane.

Response of the Nictitating Membrane to Adrenaline and Noradrenaline.—Fig. 2 shows that the intravenous injection of 0.5 to 2.5 mg. morphine reduced the response of the nictitating membrane to submaximal preganglionic stimulation (Fig. 2c) to a much greater extent than the response to supramaximal stimulation (Fig. 2a
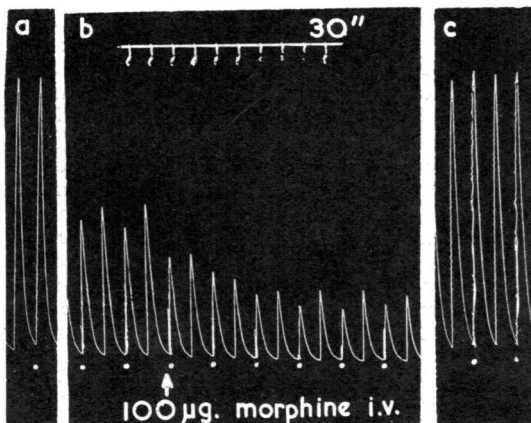

FIG. 1.—Cat, chloralose anaesthesia, 4 kg. Record of nictitating membrane. Electrical stimulation for 5 sec. twice per min., alternately applied to pre- and post-ganglionic fibres (postganglionic stimulation marked with dots). Strength of stimulation supramaximal in (a) and (c), submaximal in (b). Rate of stimulation 2/sec. Intravenous injection of 100 μg. morphine in (b). Record (c) was taken 2 min. after (b).

FIG. 2.—Cat, chloralose anaesthesia, 2 kg. Record of nictitating membrane. Preganglionic stimulation applied for periods of 5 sec. twice per min. Rate: 2/sec. Strength of stimulation: supramaximal in (a) and (d), submaximal in (c). (Upper trace.) Intravenous injections of 10, 20, and 40 μg. adrenaline (b) before and (c) after the intravenous injections of 0.5 mg. morphine. Lower trace: intravenous injections of 25 and 50 μg. noradrenaline (b) before and (c) after intravenous injection of 2.5 mg. morphine. Between the injections of 20 and 40 μg. adrenaline the drum was stopped for 5 min. (X). Record (d) was taken 2 min. after record (c).
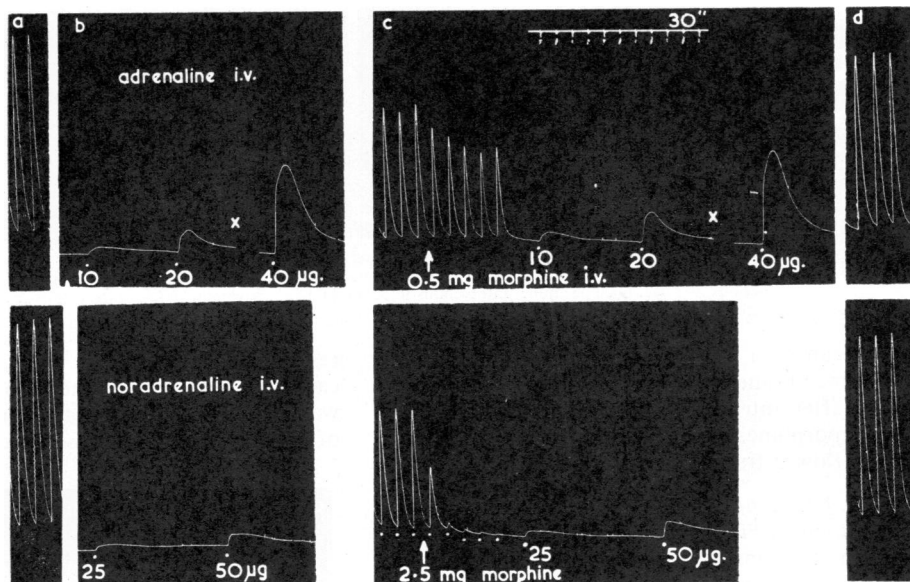
and d). Although the contractions of the nictitating membrane caused by intravenous injections of adrenaline and noradrenaline (Fig. 2b) were smaller than those caused by submaximal preganglionic stimulation, the effect of adrenaline and noradrenaline was not reduced by morphine (Fig. 2c). After intravenous injections of as much as 10 mg. morphine, the response of the nictitating membrane to intravenous injections of adrenaline and noradrenaline was slightly increased.

Stimulation of the Superior Cervical Ganglion by Nicotine-like Substances and by Potassium Chloride.—Injections of 2.5 to 10 μg. nicotine or tetramethylammonium or of 1 to 2 mg. potassium chloride into the blood supply of the superior cervical ganglion caused submaximal contractions of the nictitating membrane due to stimulation of the ganglion cells. Intravenous injections of 100 μg. and 500 μg. morphine depressed the response of the nictitating membrane to these injections to about the same extent (20% and 53% respectively) as the response of the nictitating membrane to submaximal pre- or post-ganglionic stimulation.

The response of the nictitating membrane to the intra-arterial injection of larger amounts of nicotine, tetramethylammonium or potassium chloride,
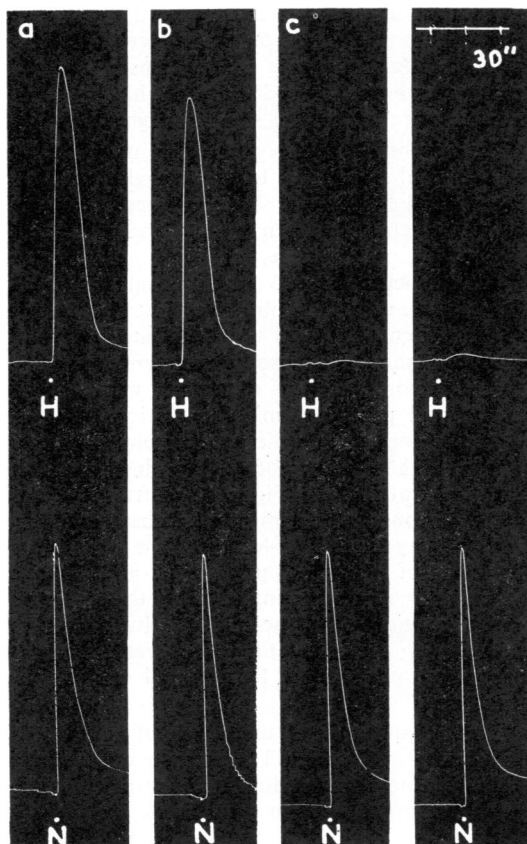

FIG. 3.—Cat, chloralose anaesthesia, 5 kg. Nictitating membranes. Intra-arterial injections to the left ganglion of 5 μg. histamine (H) and to the right ganglion of 40 μg. nicotine (N). Intravenous injection of 1 mg. morphine between (b) and (c). Time intervals of 20 min. between records.
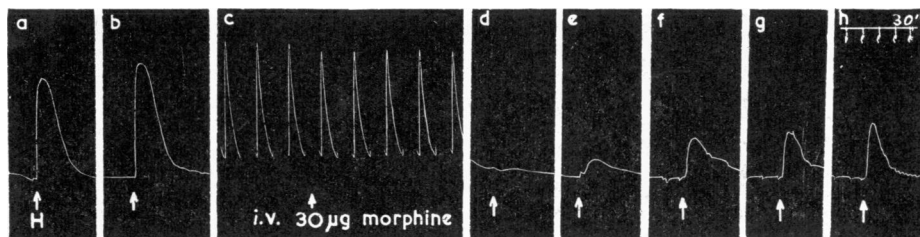
FIG. 4.—Cat, chloralose anaesthesia, 4.5 kg. Nictitating membrane. Intra-arterial injections to the ganglion of 20 μg. histamine at arrow. In (c) submaximal postganglionic stimulation applied at a rate of 2/sec. for periods of 5 sec. once per min. Intravenous injection of 30 μg. morphine in (c). Intervals of 20 min. between records.

which caused a maximal contraction of the nictitating membrane, was reduced only slightly or not at all after intravenous administration of 0.2 to 1 mg. morphine. Such an experiment is shown in Fig. 3 (lower trace).

Stimulation of the Superior Cervical Ganglion by Histamine, Pilocarpine and 5-HT.—The following observations concern a different action of morphine on the ganglion. Fig. 3 illustrates the fact that morphine abolished the response of the nictitating membrane to intra-arterial injections of 5 μg. histamine, while it did not affect the response to nicotine, although the contractions of the nictitating membrane caused by both histamine and nicotine were initially of similar height.

The response of the nictitating membrane to intra-arterial injections to the ganglion of pilocarpine and 5-HT was also abolished after intravenous injections of morphine. The amounts of morphine required were small; 5 μg. morphine usually reduced the action of these substances, and 20 to 30 μg. morphine abolished it. Fig. 4 shows an experiment in which the intravenous injection of 30 μg. morphine caused a very small reduction of the response of the nictitating membrane to submaximal postganglionic stimulation (Fig. 4c); but the response of the nictitating membrane to the intra-arterial injection of 20 μg. histamine was completely abolished (Fig. 4d). Partial recovery of the response to histamine was observed during the following 2 hr. (Fig. 4e to h). The reduction of the action of histamine, pilocarpine and 5-HT on the superior cervical ganglion was thus of much longer duration than the slight reduction of the response of the nictitating membrane to nerve stimulation caused by these small amounts of morphine.

The long duration of the action of morphine is also shown in Fig. 5. The intravenous injection of 20 μg. morphine reduced the response of the nictitating membrane to very weak submaximal preganglionic stimulation and abolished the

response to intra-arterial injections of 20 μg. pilocarpine. Recovery of the response to pilocarpine was not complete until 3 to 4 hr. after the injection of morphine. The figure also shows that mor-
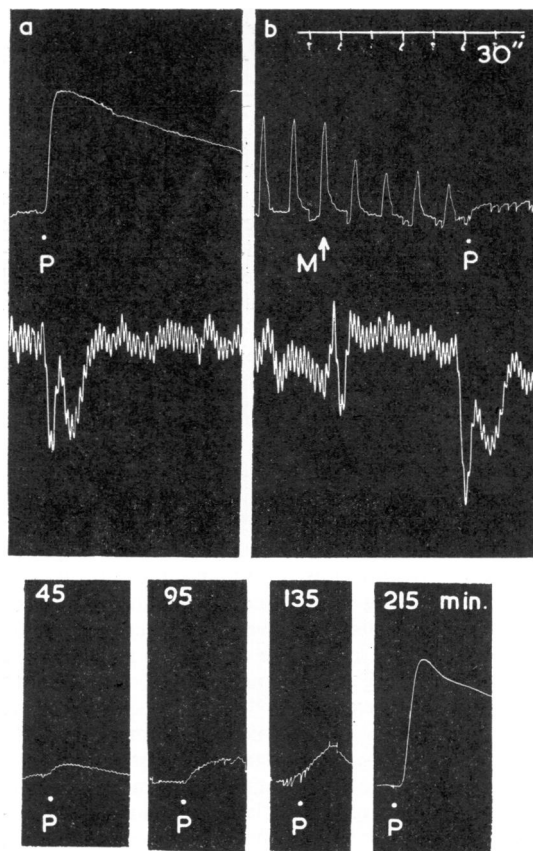

FIG. 5.—Cat, chloralose anaesthesia, 4.1 kg. Nictitating membrane and arterial blood pressure. Intra-arterial injections to the ganglion of 20 μg. pilocarpine (P). Submaximal preganglionic stimulation at a rate of 2/sec. for periods of 5 sec. twice per min. in (b). Intravenous injection of 20 μg. morphine at (M) in (b); the lower 4 records were obtained 45, 95, 135, and 215 min. after the injection of morphine.
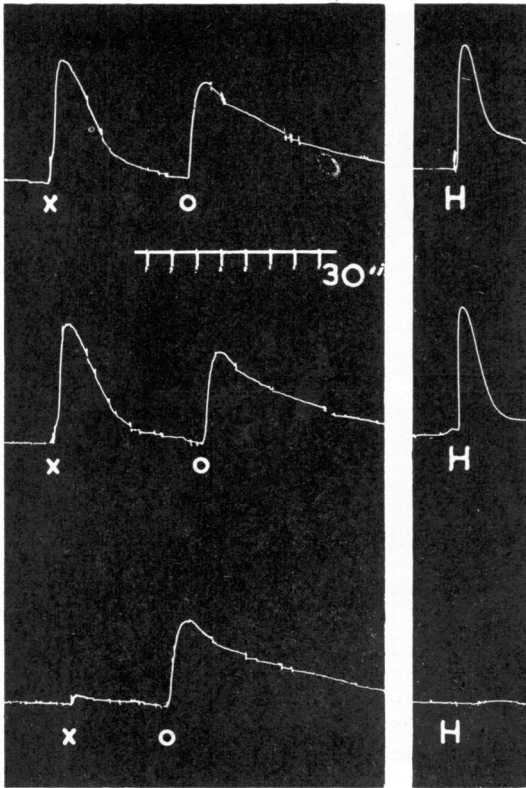
FIG. 6.—Cat, chloralose anaesthesia, 2.1 kg. Nictitating membrane. Intra-arterial injection of 10 μg. 5-HT to the ganglion (X) and to the nictitating membrane (O), intra-arterial injection to the ganglion of 5 μg. histamine (H). The upper and middle traces are before, and the lower trace is after, the intravenous injection of 50 μg. morphine. Time interval of 30 min. between traces.

phine failed to reduce the fall of blood pressure caused by pilocarpine. Likewise the depressor action of histamine was not antagonized.

In contrast to histamine and pilocarpine, 5-HT has a direct action on the smooth muscle of the nicititating membrane (Trendelenburg, 1956a). Fig. 6 shows that morphine abolished the response of the nictitating membrane caused by intra-arterial injections to the ganglion of 10 μg. 5-HT and 5 μg. histamine ; but the direct action of 5-HT (injected intra-arterially at O) on the smooth muscle of the nictitating membrane remained unchanged.

When morphine was injected intra-arterially into the blood supply of the ganglion, it was found to be active in much smaller amounts. Fig. 7 shows that the intra-arterial injection of 0.5 μg. morphine abolished the action of histamine on the

ganglion ; when, however, the same amount of morphine was injected intravenously, it failed to alter the action of histamine (Fig. 7f). Similar observations were made when 0.5 μg. morphine was injected first intravenously and then intra-arterially.

Potentiating Effects of Histamine, Pilocarpine, and 5-HT.—These three substances potentiate the response of the nictitating membrane to submaximal preganglionic stimulation, by facilitating transmission through the superior cervical ganglion (Trendelenburg, 1955, 1956a, b, and unpublished observations). Fig. 8 shows that morphine antagonized this potentiating action. 10 μg. each of pilocarpine (P), 5-HT (HT) and of histamine (H) were injected intra-arterially during intermittent submaximal stimulation of the preganglionic fibres. The intra-arterial injections were made at 20 min. intervals ; they failed to stimulate the superior cervical ganglion, but regularly caused pronounced potentiation of the response of the nictitating membrane (Fig. 8b to f). After the intravenous injection of 50 μg. morphine (between f and g), the potentiating effects of the three substances were much reduced (Fig. 8g to i), while the response of the nictitating membrane to supramaximal preganglionic stimulation was scarcely altered (compare Fig. 8a and k). In order to abolish the potentiating effect of histamine, pilocarpine and 5-HT on ganglionic transmission, larger amounts of morphine (100 to 200 μg.) had to be injected intravenously than those required for abolition of the ganglion-stimulating action of these three substances.

DISCUSSION

The present results have revealed two actions of morphine, one on some part of the postganglionic axon peripheral to the electrodes used for postganglionic stimulation, and the other on the superior cervical ganglion.
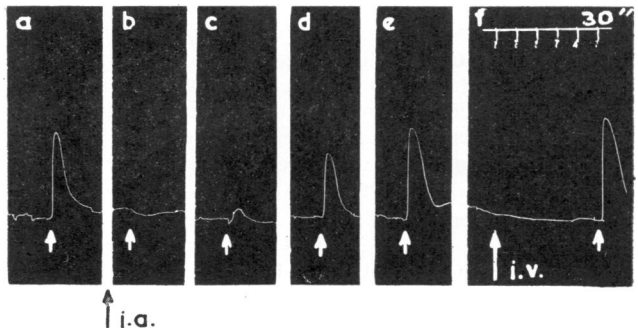

FIG. 7.—Cat, chloralose anaesthesia, 2.1 kg. Nictitating membrane. Intra-arterial injections to the ganglion of 10 μg. histamine at arrow. Injections of 0.5 μg. morphine, intra-arterially to the ganglion between (a) and (b), intravenously in (f). Time intervals of 20 min. between records.
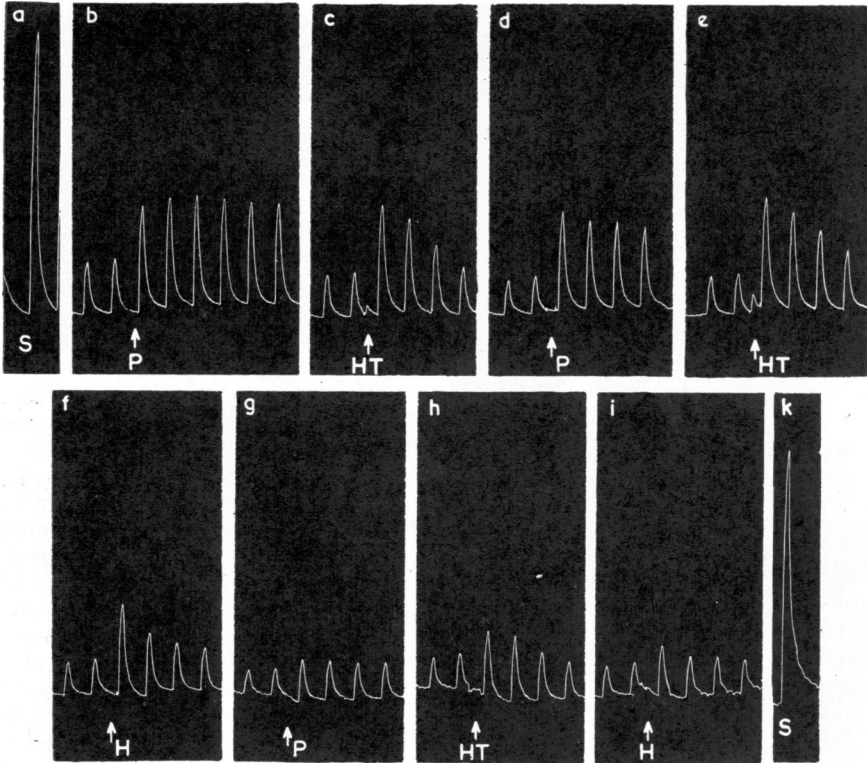
FIG. 8.—Cat, chloralose anaesthesia, 4 kg. Nictitating membrane. Preganglionic stimulation applied at a rate of 15/sec. for periods of 5 sec. twice per min. Supramaximal strength in (a) and (k), submaximal in (b) to (i). Intra-arterial injections to the ganglion of 10 μg. pilocarpine (P), 10 μg. 5-HT (HT) and 10 μg. histamine (H). Intravenous injection of 50 μg. morphine between (f) and (g). Time intervals of 20 min. between records (b) to (i).

Larger amounts of morphine (50 μg. to 2.5 mg.) depressed the response of the nictitating membrane to pre- and post-ganglionic stimulation to the same degree. Morphine thus did not block the transmission of nerve impulses through the superior cervical ganglion. Nor did it antagonize the action of nicotine-like substances and of potassium chloride on the superior cervical ganglion. The response of the nictitating membrane to intra-arterial injections of these ganglion-stimulating substances and that to preganglionic stimulation was equally reduced by morphine, provided the strength of electrical stimulation was chosen so as to produce contractions of the nictitating membrane of a similar height.

The inhibition by morphine of the response of the nictitating membrane to pre- and post-ganglionic stimulation was not due to an action of morphine on the smooth muscle of the nictitating membrane, since the direct action of adrenaline, noradrenaline and 5-HT on the nictitating membrane was not reduced.

As morphine was found to have neither a ganglion-blocking action nor a direct depressant effect on the nictitating membrane, it seemed it might act by reducing the amount of sympathetic transmitter liberated from the postganglionic nerve endings on stimulation of the pre- or post-ganglionic fibres. Such an assumption is supported by the observation (Paton, personal communication) that morphine reduces the liberation of acetylcholine from the isolated intestine on direct electrical stimulation of the wall of the intestine, which is believed to stimulate the post-ganglionic fibres embedded in the tissue.

The inhibitory action of morphine was found to be related to the height of the contractions of the nictitating membrane caused by pre- or post-ganglionic stimulation or by intra-arterial injections into the blood supply of the ganglion of nicotine-like substances or of potassium chloride. As the periods of stimulation were kept constant (5 sec.), it is likely that the height of the contraction of the effector organ was related to the amount

of sympathetic transmitter liberated per unit time. This amount was increased either by stimulating more nerve fibres with stronger currents or by increasing the frequency of stimulation from 2/sec. to 15/sec. The larger the initial contraction of the nictitating membrane the less pronounced was the depressing effect of morphine.

It has recently been pointed out that histamine, pilocarpine and 5-HT stimulate the superior cervical ganglion of the cat by combining with receptors, which differ from the acetylcholine receptors of the ganglion cells. These three substances therefore cannot be considered as having " nicotine-like " properties (Trendelenburg, 1956b). The present results provide further evidence for the view that histamine, pilocarpine and 5-HT differ from both the " nicotine-like " ganglion-stimulating substances and from potassium chloride. Small amounts of morphine, which were found to interfere neither with the liberation of sympathetic transmitter from the postganglionic nerve endings nor with the ganglion-stimulating action of nicotine-like substances and of potassium chloride, caused long-lasting depression of the response of the superior cervical ganglion to histamine, pilocarpine and 5-HT. The direct action of 5-HT on the smooth muscle of the nictitating membrane, on the other hand, was not affected by morphine. This observation agrees with recent findings reported to the British Pharmacological Society in July, 1956, by Picarelli that the guinea-pig ileum contains two types of specific tryptamine-receptors, one of which has been found to be blocked by morphine.

Previous results had shown that cocaine had an action rather similar to that of morphine, in so far as cocaine antagonized the ganglionic actions of histamine, pilocarpine and 5-HT without interfering with ganglionic transmission or the ganglion-stimulating action of nicotine-like substances and of potassium chloride (Trendelenburg, 1954, 1956a). The minimal effective dose of intravenous injections of cocaine was found to be 0.1 to 0.5 mg., while morphine was effective in doses of 5 to 20 μg. Cocaine was thus about 20 times less active than morphine when administered by the same route. Picarelli (1956) has found that cocaine antagonized the response of the isolated guinea-pig ileum to 5-HT, and that this was due to an interference with the action of 5-HT on the morphine-sensitive tryptamine receptors. Cocaine failed to antagonize the response of the ileum to 5-HT when the morphine-sensitive tryptamine receptors had been blocked by the addition of morphine to the bath. These observations provided further evidence for the view that morphine and cocaine resemble each other in their antagonistic action towards 5-HT.

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