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THE EFFECTS OF HEXAMETHONIUM AND MORPHINE ON TRANSMISSION IN THE SUPERIOR CERVICAL GANGLION OF THE RABBIT

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Morphine is known to have a depressant action on impulse transmission from postganglionic neurones to effector cells—for example, the longitudinal muscle cells of the guinea-pig ileum (Schaumann, 1955; Kosterlitz & Robinson, 1955, 1957; Paton, 1956, 1957, 1963), the sinoatrial node of the rat and the rabbit (Kosterlitz & Taylor, 1959) and the nictitating membrane of the cat (Trendelenburg, 1957; Cairnie, Kosterlitz & Taylor, 1961). While the analysis of this phenomenon is by no means complete, the following facts have been established: (1) morphine in concentrations up to 27 μM does not affect axonal conduction (Cairnie & Kosterlitz, 1962; Kosterlitz & Wallis, 1964); (2) any depressant effect morphine may have on the action of the transmitter substance on the effector cell is insufficient to explain the action of morphine on impulse transmission (Kosterlitz & Robinson, 1957; Paton, 1957; Cairnie *et al.*, 1961); and (3) it has been shown by assay that morphine has a depressant effect on the release of acetylcholine from the electrically stimulated guinea-pig ileum (Paton, 1957, 1963). This inhibition of release is present when the ileum is stimulated at low, but not at high, frequencies.

In view of these findings, it was of interest to re-examine the action of morphine on transmission from preganglionic to postganglionic neurones in the superior cervical ganglion. There is little doubt that this junction is not as sensitive to the depressant action of morphine as some postganglionic neuro-effector junctions, since neither transmission (Hebb & Konzett, 1950) nor acetylcholine release (Paton, 1957) is readily affected. The present paper gives the results of an investigation into the action of morphine on the isolated superior cervical ganglion of the rabbit in the presence of hexamethonium in a concentration which just blocked transmission. By this means it was hoped to reduce the safety factor of the synaptic pathway and thus unmask any effects morphine might have on release of transmitter or on the response of the synaptic membrane to the transmitter. The potential changes occurring in the ganglion of the rabbit have been described by Eccles (1952a,b) but some further analysis of the action of hexamethonium was necessary.

METHODS

Experimental procedure. Superior cervical ganglia were removed from rabbits anaesthetized by intravenous injection of 5 ml./kg of a 25% (w/v) solution of urethane, which was chosen because Larrabee & Posternak (1952) showed that in anaesthetic concentrations it has no depressant action on ganglionic transmission. During dissection care was taken to retain the blood supply of the ganglion for as long as possible. After excision the ganglion and part of the postganglionic trunk were desheathed under a microscope. The ganglion-bath was similar to that described by Eccles (1952a); an entry port for the introduction of drugs and a tap for draining the fluid from the bath were incorporated into the design. Adjustable electrodes were fitted to record from two of three points simultaneously—namely, from the preganglionic trunk, from the ganglion or from the internal carotid division of the postganglionic nerve (Fig. 1). The ganglion-bath was partially immersed in a water-bath, which kept the temperature of the fluid bathing the ganglion at 36 to 37° C. Water from a thermostatically controlled tank outside the recording cage was pumped to the water-bath.

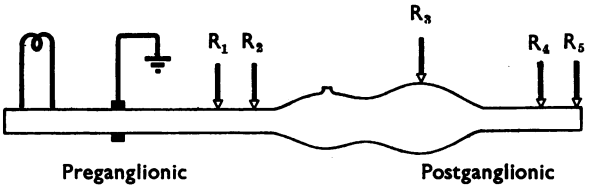

Fig. 1. The arrangement of recording and stimulating electrodes. The compound action potential from the preganglionic trunk was recorded between R_1 and R_2, that from the postganglionic trunk between R_4 and R_5. The ganglionic response was recorded between R_3 and R_5.

The bath fluid was 50 ml. of Krebs solution, bubbled with 95% oxygen and 5% carbon dioxide. Its composition was as follows (mM): NaCl 118, KCl 4.75, $CaCl_2$ 2.54, KH_2PO_4 1.19, $MgSO_4$ 1.2, $NaHCO_3$ 25 and glucose 11. The stimulating and recording electrodes were of platinum. The action potentials were led off by means of low grid-current cathode followers into capacity-coupled amplifiers with time constants of 1.5 sec. The preganglionic trunk was stimulated with single rectangular pulses, having a small biphasic component, from a Grass stimulator. The stimuli, 0.1 to 0.5 msec in duration, were isolated from earth by a radio-frequency transformer unit. The preparation was lifted from the bath fluid by tilting the bath 1 min before recording.

When the effects of morphine were studied, the preparation was exposed to 275 to 550 μM-hexamethonium, 93 μM-pentolinium or 56 μM-dihydro-β-erythroidine, concentrations which were chosen to cause a complete, or almost complete, block of transmission. The time required to obtain the maximum effect varied between 30 and 70 min. Although in a relatively small number of experiments a pure synaptic potential or N wave was obtained, it was more usual for a small residual spike to be present at the beginning of the N wave. When records showed that the shape and size of the ganglionic potential had become constant, morphine was added to the bath fluid. Any depressant effect was expressed as the reduction in the height of the "initial negative potential," which consisted of the N wave or the N wave and the residual spike. The height was measured to the peak of the residual spike or, if this became suppressed, to the peak of the N wave. To test whether a depressant effect of morphine was reversible, the morphine-analogue, nalorphine, was added or the bath fluid was replaced by fresh Krebs solution. Possible effects of morphine on the P and LN waves have not been analysed, since these potentials are small when single stimuli are applied.

The strength of the stimulus applied to the preganglionic trunk was adjusted so that it was just supramaximal for the B fibres; this was usually above threshold strength for some of the preganglionic C fibres and caused a small second deflection in the ganglionic potential. However, the second deflection was never large enough to cause difficulty in the assessment of possible effects of morphine on the initial negative potential.

Drugs. The drugs used were hexamethonium bromide, pentolinium tartrate, dihydro-β-erythroidine hydrobromide, morphine hydrochloride and nalorphine hydrobromide.

RESULTS

Action potentials in the superior cervical ganglion

The compound action potential consists of two major deflections: the first (Sa) is evoked by stimulating the preganglionic B fibres, while the second (Sb) is a response to stimulation of preganglionic C fibres (Eccles, 1952a). It follows, therefore, that the degree to which the Sa and Sb components of the ganglionic response, and the corresponding action potentials in the internal carotid nerve, are temporally separated depends upon the distance of the stimulating elecrodes from the ganglion. Unless there is a preganglionic conduction distance of at least 10 mm, the Sa and Sb deflections are fused.

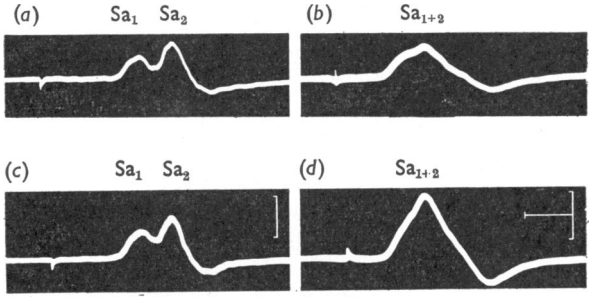

Fig. 2. The effect of varying the preganglionic and postganglionic conduction distances on the separation of the Sa_1 and Sa_2 deflections. Postganglionic records. Stimulus near maximal for Sa_1. Distance of stimulating electrodes to preganglionic pole of ganglion: (*a*) and (*b*), 30 mm; (*c*) and (*d*), 5 mm. Distance of recording electrodes to postganglionic pole of ganglion: (*a*) and (*c*), 10 mm; (*b*) and (*d*), 2 mm. Calibrations: (*a*) and (*c*), 125 μV; (*b*) and (*d*), 500 μV. Time, 10 msec.

Examination of postganglionic records taken at the distal end of the internal carotid nerve shows that the Sa deflection is due to excitation of two distinct fibre groups, Sa_1 and Sa_2, but in records taken close to the ganglion the two groups are fused (Fig. 2). Separation of the two deflections is therefore due to different conduction rates in the postganglionic fibres. An increase in the preganglionic conduction distance does not cause separation of the two deflections and this suggests that the Sa_1 and Sa_2 ganglion cells are excited simultaneously by a relatively homogeneous group of preganglionic fibres; however, the fibres evoking the Sa_2 response have a higher threshold than those responsible for the Sa_1 deflection (Fig. 3). In ganglionic records the two deflections are fused, except when the Sa_2 deflection is selectively depressed by hexamethonium, as will be described later.

When preganglionic records are monitored in order to discover a possible depressant effect of morphine on axonal conduction, the occurrence of ganglion cells or of a subsidiary ganglion within the preganglionic trunk may make interpretation difficult (Douglas & Ritchie, 1956). In some preparations this may result in a truly postganglionic component of the compound action potential recorded from the preganglionic trunk (Fig. 4, PG). The fibres responsible for this deflection have a low threshold to stimulation, characteristic of B fibres, but at the same time an apparent conduction velocity lower

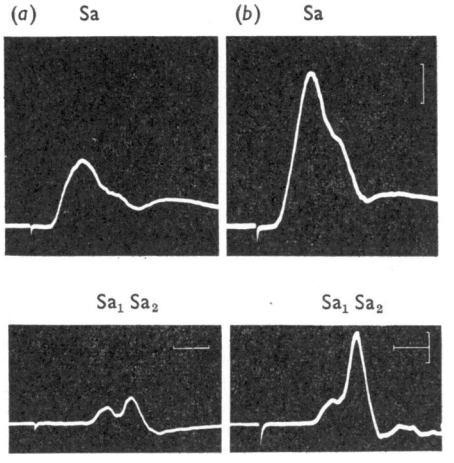

Fig. 3. Sa_1 and Sa_2 deflections in simultaneous ganglionic and postganglionic records. Upper records: ganglionic, calibration 250 μV; lower records: postganglionic, calibration 125 μV. Time, 10 msec. (*a*): stimulus near maximal for Sa_1 and submaximal for Sa_2; (*b*): stimulus maximal for both Sa_1 and Sa_2.

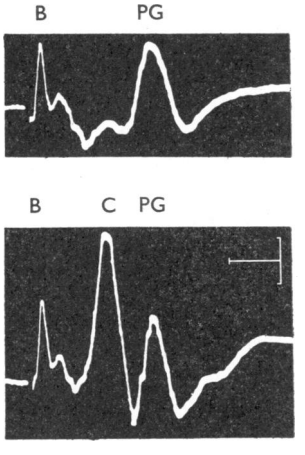

Fig. 4. The compound action potential of the cervical sympathetic trunk (R_1R_2). Upper trace: stimulus near maximal for B fibres but subthreshold for C fibres. Lower trace: stimulus maximal for C fibres. B: deflection due to B fibres; C: deflection due to C fibres; PG: deflection due to fibres which have synapsed in the preganglionic trunk between the stimulating and recording sites; note their low threshold and slow conduction rate. Calibration, 125 μV. Time, 10 msec.

than that of C fibres. The postganglionic nature of such a deflection is confirmed by the observation that it is suppressed by hexamethonium.

The effect of hexamethonium on the ganglionic potential

When hexamethonium was added to the bath fluid, the spike potential became progressively smaller, while the N wave, or synaptic potential, increased in height (Fig. 5). At the same time the positive deflection following the spike potential diminished

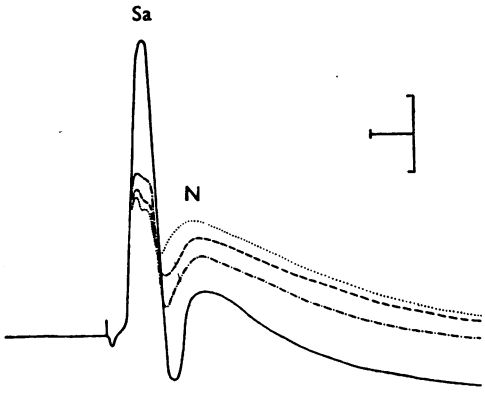

Fig. 5. The development of block due to hexamethonium. Solid line, 11 min; dots and dashes, 26 min; dashes, 42 min; dots, 70 min after addition of hexamethonium (275 μM). Stimulus near maximal for Sa. Calibration, 250 μV. Time, 20 msec.

gradually. The full effect of hexamethonium was usually established 30 to 70 min after adding the drug; the height of the initial negative potential, which consisted of the synaptic potential or of the synaptic potential and a residual spike potential, remained constant for about 2 hr, when the effect of hexamethonium started to wear off slowly.

With a concentration of hexamethonium of 275 μM, the height of the initial negative potential was reduced in twelve experiments to $24.5 \pm 1.8\%$ (mean and standard error) of the spike potential in the unblocked ganglion; with 550 μM, the corresponding values

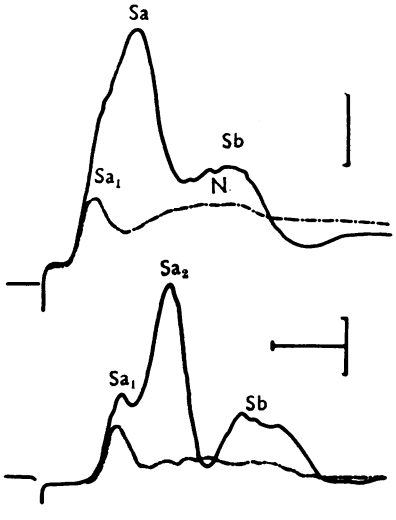

Fig. 6. The different sensitivities of the Sa_1, Sa_2 and Sb deflections to the blocking action of hexamethonium and the unmasking of the Sa_1 deflection in the ganglionic potential. Stimulus, supramaximal for Sa_1 and Sa_2 and near maximal for Sb. Upper record: ganglionic, calibration 1 mV; lower record: postganglionic, 3 mm from postganglionic pole of ganglion, calibration 250 μV. Time, 10 msec. Solid line, before hexamethonium; broken line, 23 min after addition of hexamethonium (275 μM).

obtained in three experiments were 11, 12 and 23%. Transmission was just blocked at a height of about 25% of the original spike, a figure which agrees well with that found for tubocurarine by Eccles (1952b).

Pentolinium tartrate (93 μM) was used in three experiments and gave initial negative potentials with residual spikes of 20, 28 and 40% of the original spike height. The corresponding values obtained with dihydro-β-erythroidine (56 μM) were 30, 27 and 44%.

The Sa_1 deflection is more resistant to the blocking action of hexamethonium than the Sa_2 and Sb responses, and thus a distinct Sa_1 deflection may be unmasked in ganglionic records. In the experiment shown in Fig. 6 the effects of hexamethonium were studied in postganglionic and ganglionic records. In the postganglionic record Sa_2 and Sb were almost abolished, while the Sa_1 deflection was only partly depressed; in the ganglionic record there was an N wave preceded by a residual spike which corresponds to the Sa_1 deflection of the postganglionic record.

The effect of morphine

Morphine was added to the bath fluid when the degree of block by hexamethonium had become constant. Records were usually taken 5, 10 and 15 min after the addition

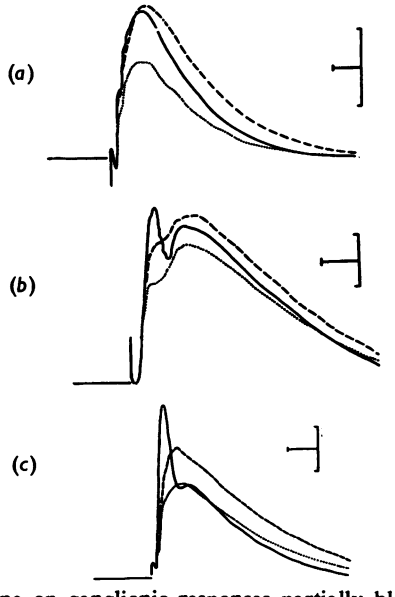

Fig. 7. The effect of morphine on ganglionic responses partially blocked by hexamethonium. (*a*): solid line, synaptic potential without residual spike, 29 min after addition of hexamethonium (550 μM), which was present throughout; dotted line, 4 min after addition of morphine (26.6 μM); dashes, 45 min after removal of morphine. Stimulus, supramaximal for Sa. Calibration, 50 μV. Time, 25 msec. (*b*): solid line, synaptic potential with residual spike, 56 min after addition of hexamethonium (275 μM), which was present throughout; dotted line, 11 min after addition of morphine (26.6 μM); dashes, 25 min after addition of nalorphine (12.7 μM). Stimulus, supramaximal for Sa. Calibration, 125 μV. Time, 25 msec. (*c*): solid line, synaptic potential with residual spike, 66 min after addition of hexamethonium (275 μM), which was present throughout; dotted line, 12 min after addition of morphine (266 μM); dashes, 20 min after addition of nalorphine (127 μM). Stimulus, near maximal for Sa. Calibration, 125 μV. Time, 25 msec.

of morphine. In some experiments nalorphine was then added, with the morphine still present, and in others the bath fluid containing morphine was replaced twice by fresh Krebs solution. In all experiments the height of the initial negative potential was plotted against time, the mean of three observations being used for each point. When the control readings before addition of morphine did not show any decline in potential height, a depressant action of morphine was easily discernible. However, if there was a spontaneous decline in the height of the control readings, depression by morphine was taken to have occurred only if adding morphine to the bath fluid produced an increase in the downward slope and if washing it out or adding nalorphine caused a change of slope in the direction of recovery.

Three examples of the effect of morphine on the ganglionic potential are shown in Fig. 7. When the application of hexamethonium led to almost complete suppression of the spike potential, morphine (26.6 μM) depressed the synaptic potential without altering the time to peak or the decay time (Fig. 7,*a*). In this experiment the synaptic potential regained height during the exposure to morphine (32 min) and recovery became complete after washing out the morphine. In other experiments a similar recovery was obtained by the addition of nalorphine (12.7 μM) in the continued presence of morphine.

In a second experiment (Fig. 7,*b*) the initial negative potential consisted of the synaptic potential and a residual Sa_1 spike, which were both depressed by morphine (26.6 μM). There was very little tachyphylaxis during the time of exposure to morphine (22 min); application of 12.7 μM-nalorphine, while morphine was still present, led to an increase in the height of synaptic potential but the spike potential was not restored to its former size. In other experiments, in which there was a larger spike potential, morphine sometimes caused an apparent increase in the height of the synaptic potential; this effect was

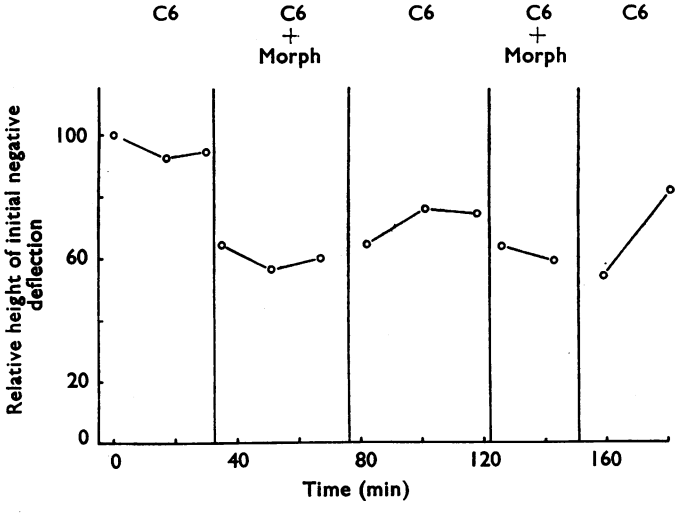

Fig. 8. The effects of successive exposures to morphine in a ganglion not quite blocked by hexamethonium. Abscissa: time in minutes (zero time is 79 min after addition of hexamethonium); ordinate: relative height of initial negative potential. Stimulus near maximal for Sa. At C6, hexamethonium (275 μM) only was present, at C6+Morph, hexamethonium and morphine (26.6 μM).

due to the fact that the suppression of the spike potential led to a decrease in post-spike positivity which had interacted with the synaptic potential.

In the third experiment (Fig. 7,*c*) a higher concentration of morphine (266 μM) was used. The residual spike potential was abolished but the apparent height of the synaptic potential remained unchanged. When nalorphine was then added to the bath to give a concentration of 127 μM, the synaptic potential increased in size but the spike did not reappear. In this experiment there was no tachyphylaxis during the exposure to morphine (25 min).

The complete course of an experiment in which morphine in a concentration of 26.6 μM was used is shown in Fig. 8. The height of the initial negative potential showed very little decline before the application of morphine, which then caused a sharp depression. After washing out the morphine the potential increased in size without reaching the original value. A second application of morphine again caused a depression and, after washing out, partial recovery was seen. This experiment was particularly successful in showing the effects of washing out morphine and applying it a second time. The main difficulties arising in this type of experiment were the impossibility of fully reversing the morphine effect by washing out the drug and the occurrence of tachyphylaxis.

The lowest concentration of morphine tested was 2.7 μM, which caused no definite depression in five experiments. In fourteen experiments 26.6 μM-morphine was applied ; the blocking drugs were hexamethonium in eleven experiments, pentolinium in one experiment and dihydro-β-erythroidine in two experiments. In eleven experiments morphine had a depressant action, the reduction in the size of the initial negative potential being $30.2 \pm 2.15\%$ (mean and standard error) and the range 21 to 38%. The three remaining experiments gave doubtful results, the depressions being 6, 7 and 12%. In four experiments, in which the morphine concentration was 266 μM, the responses were depressed by $42.0 \pm 3.64\%$.

Partial recovery of the ganglionic potential in the continued presence of 26.6 μM-morphine occurred in three out of eleven experiments ; there was no recovery in the four experiments with 266 μM-morphine. Nalorphine (12.7 μM) partly antagonized the action of 26.6 μM-morphine in six out of seven experiments, and in a concentration of 127 μM had a similar effect on 266 μM-morphine.

The results obtained with ganglionic recording were confirmed in experiments which tested the effect of morphine on the residual postganglionic action potentials of ganglia exposed to hexamethonium. Morphine in concentrations of 26.6 and 266 μM had a depressant action, which was partly antagonized by nalorphine.

The depressant effects of morphine were not due to an action on axonal conduction. In a previous paper it was shown that concentrations up to 27 μM did not affect conduction in a variety of nerve fibres (Kosterlitz & Wallis, 1964). In eight of the present experiments the action potentials of preganglionic B fibres were monitored. No depressant effects were observed for 266 μM-morphine ; however, in one out of four experiments a concentration of 2,660 μM depressed the height of the action potential by about 20%. The presence or absence of hexamethonium did not affect the result.

Nalorphine in a concentration of 25.4 μM did not depress axonal conduction or the initial negative ganglionic potential. However, in a concentration of 254 μM it had a

slight depressant action on axonal conduction, an effect which was considerable when the concentration was raised to 2,540 μM. This was not due to the fact that the bromide salt was used, since a similar concentration of hexamethonium bromide had no such effect.

Unblocked ganglia were much less sensitive to the depressant action of morphine than ganglia previously treated with blocking agents. In five experiments, in which 26.6 μM-morphine was applied, depression of the spike potential was observed only once. With a morphine concentration of 266 μM, a depression was observed in two out of three experiments.

DISCUSSION

The present investigation was undertaken to examine the possible effects of morphine on impulse transmission in an autonomic ganglion. It appears that concentrations of morphine (0.3 to 3 μM) which inhibit transmission at certain postganglionic neuro-effector junctions, for example, the guinea-pig isolated ileum (Kosterlitz & Robinson, 1957; Paton, 1957) and the isolated nictitating membrane of the cat (J. W. Thompson, 1960), have no obvious effect on transmission in the superior cervical ganglion of the rabbit.

With higher concentrations, electrophysiological changes were observed. In the normal ganglion a concentration of 27 μM exerted a depressant effect only occasionally. However, when the safety factor of transmission had been reduced by treatment of the ganglion with hexamethonium, pentolinium or dihydro-β-erythroidine, the susceptibility of the ganglion to the depressant action of morphine increased considerably but remained less than that of some of the highly sensitive postganglionic neuro-effector junctions. A concentration of 27 μM was usually effective, while the effect of 2.7 μM was doubtful.

It is certain from earlier observations (Kosterlitz & Wallis, 1964) and from present results that morphine in a concentration as high as 2,660 μM does not affect axonal conduction, but these findings do not exclude failure of conduction in the presynaptic terminals. The depression of the synaptic potentials by morphine may be due to either a decrease of transmitter release or a decreased sensitivity of the postsynaptic membrane to the transmitter, or to both. No decision can be reached on the evidence available at present.

The failure to remove the effect of morphine by washing out the drug may be due to the high concentrations used. It is well known that at postganglionic neuro-effector junctions, for example, the guinea-pig ileum, the effect of high concentrations is long-lasting, although that of a low concentration (0.3 μM) is readily reversible. Washing out morphine or adding nalorphine had a greater restorative effect on the N wave than on the residual spike. There are several possible explanations for this phenomenon, which requires further investigation.

In view of the high concentrations of morphine required to produce a depressant effect on ganglionic transmission, it cannot be stated at present whether the observations described in this paper will be of importance for an understanding of the mode of action of morphine. In this context it may be of significance that postganglionic acetylcholine receptors in smooth muscle respond to lower concentrations of acetylcholine than the acetylcholine receptors of ganglion cells—for example, 0.001 μM causes a contraction of the longitudinal muscle of the guinea-pig ileum, while 3 μM is required to produce

a discharge in the postganglionic fibres of the perfused superior cervical ganglion of the cat (Emmelin & Macintosh, 1956).

It is of interest that, in the superior cervical ganglion of the rabbit, the allyl-analogue of morphine, nalorphine, has no depressant action in concentrations up to 25.4 μM and acts as an antagonist to morphine. This is different from the action of nalorphine on the guinea-pig ileum, in which it inhibits the response of the longitudinal muscle to coaxial electrical stimulation as powerfully as morphine (Paton, 1957 ; Gyang, personal communication).

The analysis of the action of hexamethonium resulted in the finding that the first main deflection, Sa, in the ganglionic potential and the postganglionic action potential in the internal carotid nerve consisted of two distinct groups, Sa_1 and Sa_2. The main electrophysiological difference between the two subgroups is the faster postganglionic conduction rate of the fibres responsible for Sa_1. Further, the ganglion cells giving rise to the Sa_1 deflection are more resistant to the action of blocking agents ; this observation agrees with the findings of Volle (1962), who recorded from the thyroid branch of the postganglionic nerve of the superior cervical ganglion of the cat and showed that the fast-conducting neurones are more resistant to the blocking action of hexamethonium, tetraethylammonium and tubocurarine than the slow-conducting neurones. Earlier experiments with hexamethonium (Morrison & Paton, quoted by Paton & Zaimis, 1952) and botulinum toxin (Ambache, 1952) indicated that the ganglion cells innervating the nictitating membrane are more resistant to substances affecting transmission than those innervating the pupil.

SUMMARY

1. It has been confirmed that, in the isolated superior cervical ganglion of the rabbit, the first main ganglionic action potential (Sa) is evoked by stimulating preganglionic B fibres and the second (Sb) by stimulating preganglionic C fibres.

2. The Sa group of ganglion cells can be subdivided into Sa_1 and Sa_2 ; the postganglionic fibres arising from the Sa_1 cells have a higher conduction velocity than those arising from Sa_2 cells. Transmission to Sa_1 cells is more resistant to the action of hexamethonium than transmission to Sa_2 and Sb cells.

3. Morphine, applied to ganglia in which transmission is just blocked by hexamethonium (275 to 550 μM), depresses the height of the synaptic potential ; any residual spike potential is also depressed. The minimum effective concentration of morphine is about 25 μM, which is considerably higher than that found to depress transmission across postganglionic neuro-effector junctions (0.3 to 3 μM). Unblocked ganglia are more resistant to the action of morphine than ganglia previously treated with hexamethonium.

4. It is difficult to reverse the effect of morphine by washing it out, but nalorphine antagonizes the action of morphine.

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