Changes in the responsiveness of the colon and vas deferens to nerve stimulation and injected transmitters in the pithed rat following chronic morphine treatment

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In experimental animals, chronically treated with morphine, drug withdrawal causes hyperexcitability which is probably central in origin but is partly mediated via the peripheral autonomic nervous system. There is some evidence that this hyperexcitability persists even in isolated tissues (Kaymakcalan & Temelli, 1964). This investigation sought to confirm the existence of morphine-induced hyperexcitability in peripheral autonomic neuro-effector systems and to determine if the phenomenon survived destruction of the central nervous system.

Male Wistar rats (200–300 g) were divided into three groups. One group received daily injections of morphine (4–400 mg/kg subcutaneously), supplemented by morphine in the drinking water. These rats received on average an oral dose of 2 mg/kg daily. The second (control) group received daily injections of normal saline (2 ml/kg subcutaneously). The third group was untreated.

The spinal autonomic outflows to the colon and the vas deferens were stimulated in pithed rats using the movable electrode technique (Gillespie, MacLaren & Pollock, 1969). The sensitivities of these two organs to nerve stimulation were measured. For comparison, the sensitivities of the colon to injected acetylcholine (0·01–10 mg/kg intravenously) and of the vas to injected noradrenaline (0·001–1 mg/kg intravenously) were also measured.

In saline-treated and untreated rats, acutely administered morphine (3–12 mg/kg intravenously) inhibited the responses of the colon and the vas to nerve stimulation but not to the exogenously administered transmitters. In rats chronically treated with morphine the responsiveness of the test organs to nerve stimulation depended upon the time interval between the last injection of morphine and examination. In rats examined less than 1 h after the last injection, neither the colon nor the vas responded to nerve stimulation. When examined 18 h after the last injection, the responses of the test organs to nerve stimulation were apparently similar to those of control and untreated rats. However, latent changes in the sensitivities of these organs could be revealed by acutely administered nalorphine (250 mg/kg intraperitoneally), which uncovered a marked hypersensitivity in both organs to nerve stimulation.

REFERENCES

GILLESPIE, J. S., MACLAREN, A. & POLLOCK, D. (1969). A method of stimulating different segments of the sympathetic and parasympathetic outflows from the spinal cord in the pithed rat. *Br. J. Pharmac.*, **37**, 513*P*.

KAYMAKCALAN, S. & TEMELLI, S. (1964). Response of the isolated intestine of normal and morphine-tolerant rats to morphine and nalorphine. *Archs int. Pharmacodyn. Thér.*, **151**, 136–141.

Potentials in isolated rat superior cervical ganglia produced by nicotine

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Depolarization of isolated rat ganglia by acetylcholine or carbachol is followed by hyperpolarization when the drug is washed out (Pascoe, 1956; Brown, 1966). This hyperpolarization probably results from active extrusion of accumulated intra-

cellular Na^+ (Kosterlitz, Lees & Wallis, 1968, 1970; Brown, Brownstein & Scholfield, 1969).

However, such an after-hyperpolarization does not usually occur when nicotine is the depolarizing agent (Fig. 1a), even though Na^+ entry during the preceding depolarization (measured by ^{24}Na uptake and total Na) is similar to that occurring during carbachol depolarization.

This difference may stem from prolonged effects of nicotine on *passive* ionic movements. Rang & Ritchie (1968) have shown that post-tetanic hyperpolarization generated by Na^+-extrusion is opposed by passive ionic movements. Conceivably, the increased membrane permeability presumably responsible for the depolarization might persist for a sufficient time after removing nicotine from the bath fluid to permit a complete short-circuit of the Na^+-potential.

To test this, we tried to curtail the permeability increase by adding hexamethonium to the washout fluid. Nicotine depolarization was then succeeded by a large after-hyperpolarization (Fig. 1b).

Measurement of the efflux of radioactivity into non-radioactive Krebs solution from ganglia incubated for 4 min in 10^{-5} g/ml ^3H-nicotine or ^3H-acetylcholine (with physostigmine) indicated that the ganglionic content of nicotine declined more slowly than that of acetylcholine (and so presumably of carbachol).

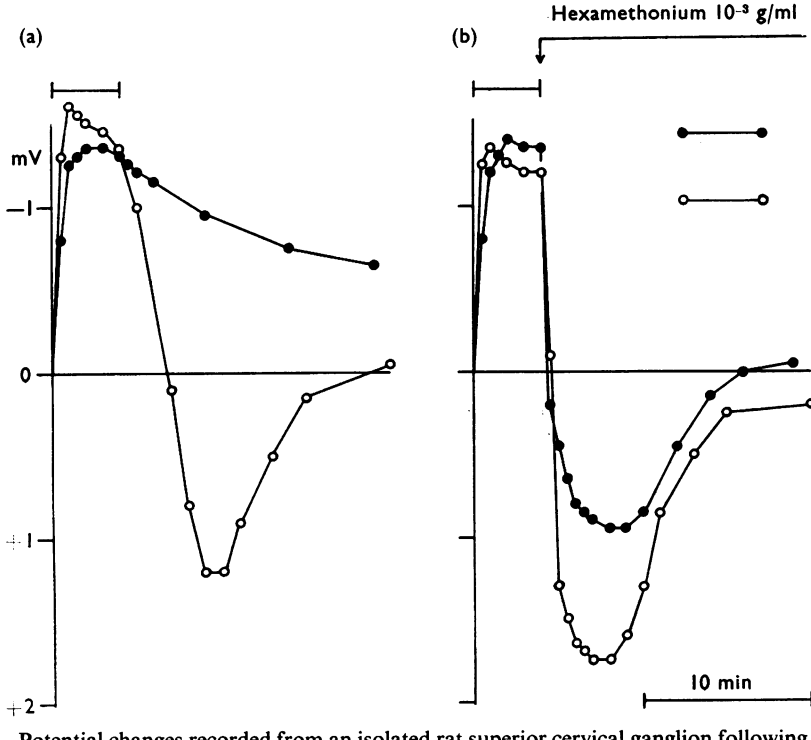

FIG. 1. Potential changes recorded from an isolated rat superior cervical ganglion following application of nicotine 10^{-5} g/ml (●——●) or carbachol chloride $3{\cdot}2\times10^{-5}$ g/ml (○——○) for 4 min. as shown by the bars above the records. Ordinates, ganglionic potential change with respect to the post-ganglionic trunk (depolarization upwards). Abscissae, time. In (a), the drugs were washed out with normal Krebs solution, in (b) with Krebs solution containing 10^{-3} g/ml hexamethonium bromide. Recording methods and experimental conditions were those described by Brown (1966).

Nicotine is accumulated intracellularly in ganglia (Brown, Halliwell & Scholfield, 1969). This might form a slowly clearing reservoir sustaining extracellular nicotine levels and consequent receptor activation during washout.

A pertinent question arising from this work is whether similar explanations, rather than postulation of separate hyperpolarizing receptors, might account for some other instances of increased ganglionic hyperpolarization following nicotinic blocking agents.

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REFERENCES

BROWN, D. A. (1966). Depolarization of normal and preganglionically denervated ganglia by stimulant drugs. *Br. J. Pharmac. Chemother.*, **26**, 511–520.

BROWN, D. A., BROWNSTEIN, M. J. & SCHOLFIELD, C. N. (1969). On the nature of the drug-induced after-hyperpolarization in isolated rat ganglia. *Br. J. Pharmac.*, **37**, 511–513*P*.

BROWN, D. A., HALLIWELL, J. V. & SCHOLFIELD, C. N. (1969). Nicotine uptake by isolated rat ganglia. *Br. J. Pharmac.*, **37**, 510–511*P*.

KOSTERLITZ, H. W., LEES, G. M. & WALLIS, D. I. (1968). Resting and action potentials recorded by the sucrose-gap. *J. Physiol., Lond.*, **195**, 39–53.

KOSTERLITZ, H. W., LEES, G. M. & WALLIS, D. I. (1970). Further evidence for an electrogenic sodium pump in a mammalian sympathetic ganglion. *Br. J. Pharmac.*, **38**, 464–465*P*.

PASCOE, J. E. (1956). The effects of acetylcholine and other drugs on the isolated superior cervical ganglion. *J. Physiol., Lond.*, **132**, 242–255.

RANG, H. P. & RITCHIE, J. M. (1968). On the electrogenic sodium pump in mammalian non-myelinated nerve fibres and its activation by various external cations. *J. Physiol., Lond.*, **196**, 183–222.

The effect of drug pretreatment on synaptic activity in *Helix* brain

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The effect of pretreatment with a series of compounds on two inhibitory dopamine pathways, one excitatory cholinergic pathway and one excitatory 5-hydroxytryptamine (5-HT) pathway in the snail brain has been investigated. One dopamine pathway produced an inhibitory postsynaptic potential (ipsp) while the other produced an inhibition of long duration (ILD) lasting for several seconds (Kerkut, Horn & Walker, 1969). Both excitatory pathways produced excitatory postsynaptic potentials (epsps) following presynaptic stimulation.

Compounds were injected into the snail haemocoel in 0·2 ml distilled water. Stimuli were applied to the relevant nerve at a frequency of 1·2 Hz. The voltage was selected to give a unitary monosynaptic response. The size of the potential after one stimulus is termed the initial height. Following repetitive stimulation the epsp declined while the ipsp increased to a constant height, the final height. The drug effect on initial and final heights and on the number of stimuli required to give the final height are recorded in Table 1.

Para-chlorophenylalanine, α-methyl-5-hydroxytryptophan or reserpine reduced the initial and final heights of the 5-HT epsp. *Para*-chlorophenylalanine also decreased the number of stimuli required to give the final height. Hemicholinium reduced the initial and final heights of the cholinergic epsp. The time taken for the epsp amplitude to fall to a constant amplitude was also reduced.

Alpha-methyl-3-4-dihydroxyphenylalanine, α-methyl-*p*-tyrosine or 6-hydroxydopamine reduced the initial and final heights of the ipsp and the ILD height. α-Methyl-3-4-dihydroxyphenylalanine and α-methyl-*p*-tyrosine also reduced the final height