carbon dioxide (pH 7·4), and containing $^{3}$H-nicotine. Tritium was extracted by dissolving the ganglia in methanolic KOH solution and counted: $^{3}$H-nicotine was not metabolized by isolated ganglia. Ganglionic water content was determined from the wet and dry weights, and extracellular space measured using $^{3}$H-mannitol. Ganglion depolarization was measured using the moving-fluid electrode technique (Pascoe, 1956).

After 30 min incubation in $^{3}$H-nicotine (5 $\mu$g/ml.), the intracellular/extracellular fluid concentration ratios (means ± S.E. of means) in the ganglia were: sympathetic, 7·8 ± 0·22; nodose, 6·1 ± 0·13. The intracellular concentration in the sympathetic ganglion was 30 ± 4% greater than that in the nodose ganglion, in reasonable agreement with previous *in vivo* observations (Brown *et al.*, 1969).

A full blocking concentration of hexamethonium (1 mg/ml.) reduced the uptake of nicotine by the sympathetic ganglion by up to 19%, without modifying nicotine uptake by the nodose ganglion. This effect of hexamethonium was only seen when strongly-depolarizing concentrations of nicotine were used. This suggests that depolarization can augment the uptake of nicotine by sympathetic ganglion cells.

Measurement of the partition of a weak acid, $^{14}$C-5,5-dimethyl-2,4-oxazolidinedione (DMO, Waddell & Butler, 1959) between intra and extracellular fluids indicated that the overall intracellular pH in the sympathetic ganglion was reduced by 0·16 ± 0·01 pH units during application of nicotine (5 $\mu$g/ml.), with no significant change of nodose ganglion cell or bath fluid pH. Since nicotine probably penetrates cell membranes as the unionized base (Weiss, 1966), this reduction of intracellular pH may play a part in increasing cellular uptake of nicotine, by increasing the degree of ionization. However, the pH gradient between extra and intracellular fluids in the absence of ganglion cell depolarization (0·003 pH units) is not sufficient to account for the high intracellular nicotine concentrations in non-depolarized ganglia.

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## On the nature of the drug-induced after-hyperpolarization in isolated rat ganglia

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The depolarization of isolated rat or rabbit superior cervical ganglia produced by acetylcholine or carbachol is followed, on washing out the depolarizing agent, by a

pronounced after-hyperpolarization (Pascoe, 1956; Brown, 1966; Kosterlitz, Lees & Wallis, 1968).

Post-tetanic hyperpolarization in mammalian non-myelinated nerve fibres (Rang & Ritchie, 1968), and in frog sympathetic ganglion cells (Nishi & Koketsu, 1968) has been ascribed to electrogenic $Na^+$ extrusion: that is, $Na^+$ extrusion incompletely balanced by $K^+$ influx. From observations on the effect of $K^+$ ions, Kosterlitz *et al.* (1968) suggested that active $Na^+$ intrusion might also account for the drug-induced after-hyperpolarization in rabbit ganglia. The present experiments on isolated rat ganglia were undertaken to examine this possibility a little further.

Superior cervical ganglia dissected from urethane-anaesthetized rats were de-sheathed and suspended in Krebs solution at room temperature, equilibrated with 95% oxygen/5% carbon dioxide mixture. Potential changes were recorded using the moving-fluid electrode technique (Pascoe, 1956). Carbachol ($3{\cdot}2\times10^{-5}$ g/ml.) was added to the bathing fluid for 4 min, then washed out. This produced a depolarization of 2–5 mV and an after-hyperpolarization of 2–4 mV.

Dinitrophenol (0·2 mM) or ouabain (0·14 mM) reversibly depressed the after-hyperpolarization without affecting the depolarization. This suggests an involvement of oxidative phosphorylation and adenosinetriphosphatase in the production of the after-hyperpolarization. Dependence on metabolism is also implicit in the observation of Pascoe (1956) that the after-hyperpolarization is reduced at low temperatures.

The after-hyperpolarization was reduced by replacing the $K^+$ in the Krebs solution with $Cs^+$, which is less effective than $K^+$ in stimulating the $Na^+$ pump in nerve fibres (Baker & Connelly, 1966; Rang & Ritchie, 1968).

Replacement of $Cl^-$ with the impermeant anion isethionate strongly enhanced the after-hyperpolarization without increasing the depolarization: by reducing intracellular $Cl^-$, isethionate reduces the " short-circuit " by $Cl^-$ efflux of the potential created by $Na^+$ extrusion (Rang & Ritchie, 1968).

These results indicate that the ganglionic after-hyperpolarization is closely similar to post-tetanic hyperpolarization in non-myelinated nerve fibres in both its metabolic requirements (Ritchie & Straub, 1957) and ionic dependence (Rang & Ritchie, 1968), and are compatible with the view that it results from electrogenic extrusion of $Na^+$ ions accumulated during depolarization.

The hyperpolarization of frog sympathetic ganglion cells observed after repetitive preganglionic nerve stimulation is suppressed by atropine (Tosaka, Chichubu & Libet, 1968). In contrast, neither atropine nor hyoscine affected carbachol-induced after-hyperpolarization in rat ganglia.

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## A method of stimulating different segments of the sympathetic and parasympathetic outflows from the spinal cord in the pithed rat

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A simple technique (Gillespie & Muir, 1967) permitted electrical stimulation of the entire sympathetic outflow from the spinal cord in the pithed rat. This technique has now been refined to permit electrical stimulation of different segments of the autonomic outflow. Responses have been obtained from the adrenals, the bladder, the blood vessels, the colon, the heart and the vas deferens.

Rats (200–250 g) were anaesthetized with halothane and respired artificially. Each animal was pithed by first inserting a short steel tube (13 S.W.G.) through the orbit and into the foramen magnum. Through this tube were passed successively a teflon tube and inside that a fine steel tube (26 S.W.G.) which was extruded at the sacral end to complete the pithing. A steel rod, inserted under the skin behind the skull and pushed down between the vertebral column and the skin, acted as an indifferent electrode. The level of stimulation was determined by varying the depth of insertion of the shielding teflon tube. The number of segments stimulated was regulated by altering the length of central steel electrode protruding. These levels and lengths were checked by radiography. (+)-Tubocurarine (1 $\mu$g/g) was given to reduce skeletal muscle twitches.

Stimulation of the cranial outflow produced vagal slowing of the heart which was abolished by atropine. Stimulation of the sacral outflow produced an increased bladder pressure and, less frequently, a rise in intra-colonic pressure. These effects were achieved without affecting either the blood pressure or the heart rate, indicating specific stimulation of the sacral parasympathetic outflow. The colonic response, which was less reproducible than the bladder response and rapidly diminished after repeated stimulation, was reduced by atropine. The bladder response was little affected by atropine (1 $\mu$g/g), hyoscine (2 $\mu$g/g) or ophenadrine (10 $\mu$g) but was reduced by an additional dose of (+)-tubocurarine (2 $\mu$g/g) and increased by eserine.

Stimulation in the lower thoraco-lumbar region produced sympathetic responses; an increase in the hind limb perfusion pressure with little effect on the systemic blood pressure and none on the heart rate. Stimulation at a higher level in the column produced longitudinal contractions and increased resistance to perfusion of the vas deferens without affecting the cardiovascular system. These responses were similar to those produced by intravenously administered noradrenaline but were blocked