Brit. J. Pharmacol. (1966), **26**, 511–520.

DEPOLARIZATION OF NORMAL AND PREGANGLIONICALLY DENERVATED SUPERIOR CERVICAL GANGLIA BY STIMULANT DRUGS

BY

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(*Received September* 17, 1965)

Evidence has recently been obtained to suggest that degenerative section of the preganglionic cervical sympathetic nerve trunk does not increase, but rather reduces, the sensitivity of the cat superior cervical ganglion to carbachol and other cholinesterase-resistant stimulant drugs (Volle & Koelle, 1961). In this study the postganglionic nerve discharges following close-arterial injection of stimulant drugs were recorded *in vivo*.

The object of the present investigation was to find out if preganglionic denervation produced comparable changes in the depolarizing action of drugs. Depolarization can be measured conveniently in isolated ganglia by the method of Pascoe (1956) and Mason (1962). Using this technique, the depolarizing actions of acetylcholine and carbachol on isolated normal and denervated rat superior cervical ganglia, and the action of carbachol on isolated normal and denervated kitten ganglia, have been studied. Kitten ganglia, rather than adult cat ganglia, were used in the hope that, by virtue of their smaller size and lower connective tissue content, rapid inward diffusion of drugs and oxygen would be facilitated.

METHODS

The superior cervical ganglion, with attached pre- and post-ganglionic nerve trunks, was isolated from rats (anaesthetized with 1.5 g/kg of urethane intraperitoneally) or from 5- to 10-week-old kittens (anaesthetized with 30 mg/kg of pentobarbitone sodium intraperitoneally), and placed in cold Krebs' solution equilibrated with 95% oxygen/5% carbon dioxide mixture. The connective tissue sheath surrounding the ganglion was removed and the preparation mounted vertically, with the postganglionic nerve uppermost, in a 50 ml. bath of oxygenated Krebs' solution enclosed in an earthed metal box.

Ganglionic potential changes were recorded in the manner described previously by Pascoe (1956) and Mason (1962). Two silver/silver chloride/agar-saline electrodes were used. The upper electrode was placed against a silk thread attached to the cut postganglionic nerve trunk. The lower electrode was placed in the bath fluid. The electrodes were connected through a direct-coupled pre-amplifier to the Y-plates of an oscilloscope. The potential difference across the electrodes reflected that between the postganglionic trunk and the region of the preparation in contact with the meniscus of the fluid. The fluid meniscus was moved along the length of the preparation by lowering the bath,

and this movement transposed linearly to the X-plates of the oscilloscope with the aid of a potentiometer, so that the potential distribution along the preparation with respect to that of the postganglionic trunk could be recorded.

To record ganglionic action potentials the fluid meniscus was lowered until the preparation was in air and the lower electrode placed on the ganglion or preganglionic nerve trunk as desired. Single stimulating shocks of 0.1 msec duration were applied to the preganglionic nerve trunk from an electronic stimulator (Bell, 1957) through an isolating transformer and bipolar platinum electrodes.

All experiments were performed at room temperature: the depolarizing action of acetylcholine is not materially affected by temperature changes within the region of 10 to 30° C (Pascoe, 1956). Drugs were made up to the required concentration in oxygenated Krebs' solution, and added to the bath from a reservoir enclosed in the same metal box as the preparation.

" Denervated " ganglia were obtained by sectioning the preganglionic cervical sympathetic trunk under pentobarbitone anaesthesia, using full aseptic precautions, 10 to 30 days prior to isolation. Degeneration of the preganglionic nerve fibres was ascertained in kittens by relaxation of the corresponding nictitating membrane, and in rats by narrowing of the palpebral fissure. Absence of a conducted preganglionic action potential served as confirmation that the fibres had degenerated.

Drugs.—Doses of the following drugs refer to the weight of the salt: acetylcholine chloride, carbachol chloride, and physostigmine salicylate (eserine salicylate). Doses of dyflos (di-isopropylfluorophosphonate, DFP) give the weight of the pure compound.

RESULTS

Isolated rat superior cervical ganglia

Acetylcholine solution, in concentrations of 10^{-5} g/ml. or more, produced a negative potential shift at the ganglion with respect to the potential of the postganglionic trunk (ganglionic depolarizaion), which was usually greatest when the fluid meniscus was at the preganglionic pole of the ganglion. Depolarization attained a maximal level within 1 to 2 min of adding the acetylcholine. After washing out the acetylcholine a pronounced ganglionic " after-hyperpolarization " occurred, the ganglion becoming strongly positive to the postganglionic trunk. As the concentration of acetylcholine was increased within the range 10^{-5} to 10^{-3} g/ml., so the magnitude of both depolarization and after-hyperpolarization increased, and the time elapsing between removal of acetylcholine solution and the peak after-hyperpolarization became longer (Fig. 1). These findings accord with those reported previously (Pascoe, 1956 ; Mason, 1962).

The rat superior cervical ganglion was about 30 times more sensitive to carbachol than to acetylcholine. The time-courses of depolarization and after-hyperpolarization following application of equi-effective depolarizing concentrations of acetylcholine and carbachol differed (Fig. 2). With carbachol the time required to attain a steady level of depolarization was about twice that required with acetylcholine. The after-hyperpolarization following removal of carbachol was smaller and slightly later than that observed after washing out acetylcholine. These differences could not be ascribed to the effects of cholinesterase because the speed of depolarization following application of acetylcholine was unaffected by physostigmine, and the difference in the after-hyperpolarization seen with acetylcholine and carbachol was exaggerated in the presence of physostigmine (Fig. 2,*b*).

Sensitivity of normal and denervated ganglia.—The sensitivity of rat ganglia to the depolarizing action of acetylcholine and carbachol was measured by constructing dose-

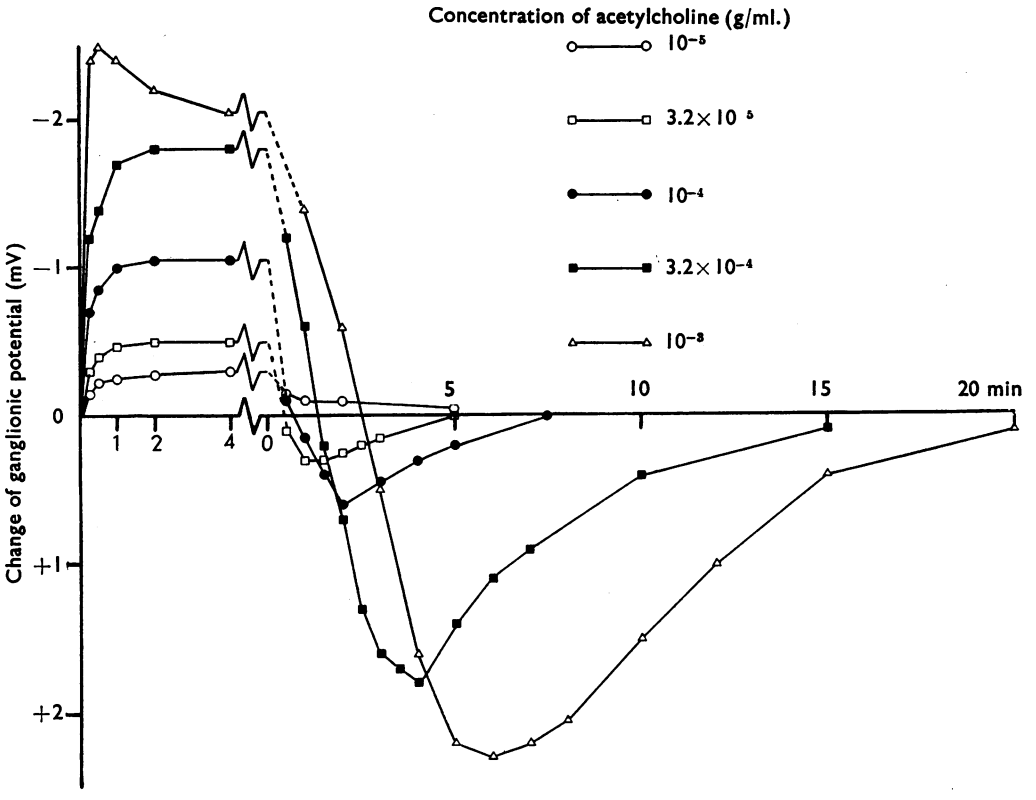

Fig. 1. Effects of increasing concentrations of acetylcholine on the potential difference between the ganglion and postganglionic nerve trunk of an isolated rat superior cervical ganglion preparation (normal). Ordinate, potential change (mV). Abscissa, time after addition and (after break) removal of acetylcholine solution (min).

response curves and determining the ED50 therefrom. In each experiment, increasing concentrations of one of the drugs were applied for 4 min at 45 min intervals, and the peak level of depolarization at the region of maximal potential shift was measured. Results from preparations in which the maximal depolarization was less than 2 mV were rejected, on the basis that the low level of depolarization suggested excessive damage. Results with 2 normal ganglia and 1 denervated ganglion were discarded for this reason.

The mean ED50 values obtained with acetylcholine and carbachol on normal and denervated ganglia are listed in Table 1. There was no significant difference between the sensitivities of normal and denervated ganglia to either of the drugs. The size of the maximal depolarization was not materially altered by denervation. The relation between the concentration of drug and the height of the depolarization was also similar in normal and denervated ganglia, as shown in Fig. 3. The time-courses of the responses of denervated ganglia to acetylcholine and carbachol closely resembled those described above for normal ganglia.

The responses of rat ganglia to acetylcholine or carbachol were usually maintained very well up to at least 48 hr after isolation. In one preparation the depolarizing action

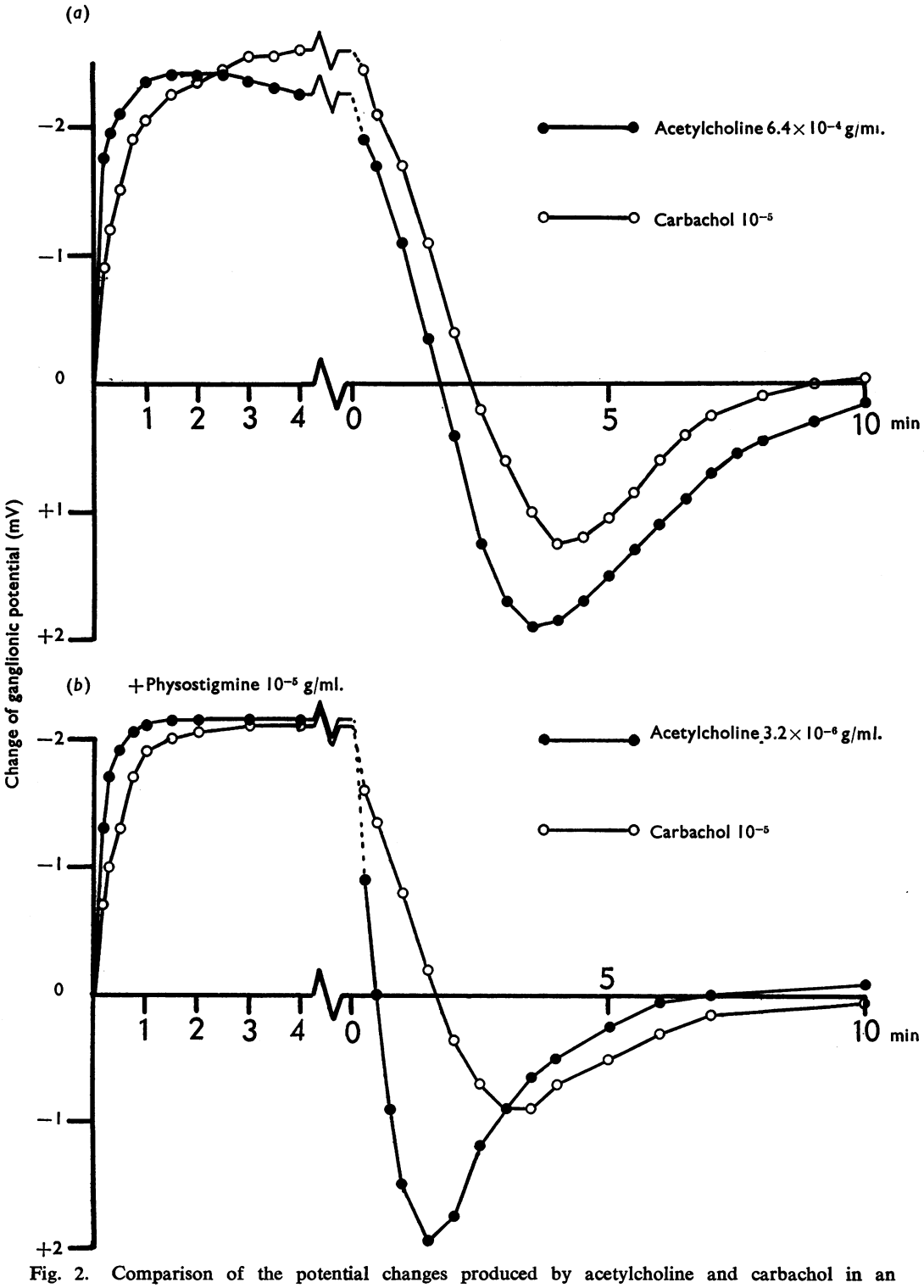

Fig. 2. Comparison of the potential changes produced by acetylcholine and carbachol in an isolated normal rat superior cervical ganglion preparation (*a*) before and (*b*) in the presence of physostigmine solution (10^{-5} g/ml.). Ordinates, change in the potential difference between the ganglion and postganglionic nerve trunk (mV). Abscissae, time after addition and (after break) removal of acetylcholine or carbachol (min).

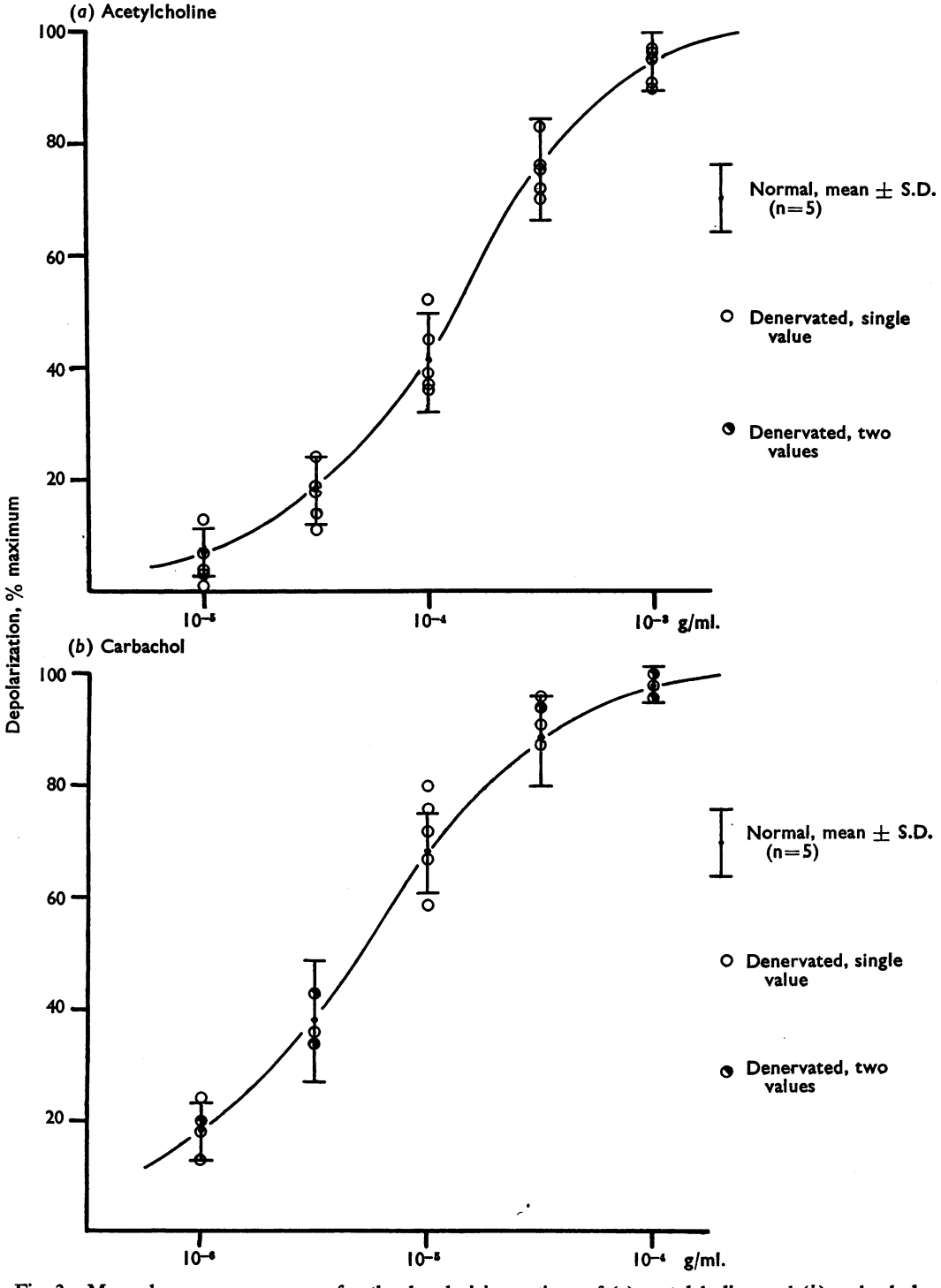

Fig. 3. Mean dose–response curves for the depolarizing actions of (*a*) acetylcholine and (*b*) carbachol on isolated rat superior cervical ganglia. Ordinates, ganglionic depolarization expressed as % of the maximal depolarization obtained. Abscissae, concentration of drug (g/ml.). The curves are drawn through the means of the values obtained using normal ganglia. Superimposed are individual values obtained with preganglionically denervated ganglia.

TABLE 1

DEPOLARIZATION OF NORMAL AND CHRONICALLY DENERVATED GANGLIA: SUMMARY OF RESULTS

		Normal ganglia			Denervated ganglia			Difference of means (*P* values)	
Species	Drug	No. of expts.	ED50 g/ml. mean±S.E.	Max. depoln. mV mean ±S.E.	No. of expts.	ED50 g/ml. mean±S.E.	Max. depoln. mV mean ±S.E.	ED50	Max. depoln.
Rat	Acetylcholine	8	$1{\cdot}45\times10^{-4}$ $\pm0{\cdot}16\times10^{-4}$	3·1±0·3	5	$1{\cdot}3\times10^{-4}$ $\pm0{\cdot}12\times10^{-4}$	2·8±0·4	0·5/0·6	0·5/0·6
	Carbachol	5	$5{\cdot}2\times10^{-6}$ $\pm0{\cdot}6\times10^{-6}$	2·9±0·5	5	$5{\cdot}0\times10^{-6}$ $\pm0{\cdot}8\times10^{-6}$	2·9±0·4	0·8/0·9	—
Kitten	Carbachol	5	$9{\cdot}4\times10^{-6}$ $\pm0{\cdot}9\times10^{-6}$	1·7±0·2	3	$9{\cdot}1\times10^{-6}$ $\pm0{\cdot}5\times10^{-6}$	2·0±0·6	0·8/0·9	0·2/0·3

of carbachol was measured at intervals over a period of 11 days after excision, the ganglion being maintained in oxygenated Krebs' solution at 4° C between tests—an *in vitro* "denervation." The transmitted action potential had diminished to less than half its original height by the second day following excision of the ganglion, and was absent on the third day. The depolarization produced by carbachol remained constant for the first 4 days after isolation (Fig. 4). Subsequently, the maximal depolarization obtainable declined, probably reflecting the death of a proportion of the ganglion cells. However, the responses to low concentrations of carbachol did not substantially diminish, indicating no alteration of the sensitivity of the remaining viable ganglion cells to carbachol during the 11 days' isolation.

Attempts were made to measure the "threshold" depolarizing concentration of carbachol in isolated rat ganglia. Taking a depolarization of 50 μV as a "threshold" response, there was no indication of any marked alteration of the threshold concentration of carbachol following denervation: in 2 experiments with normal ganglia, threshold concentrations were 9 and 18×10^{-9} g/ml. of carbachol, and in 2 denervated ganglia 4 and 7×10^{-9} g/ml.

Effects of anticholinesterase agents.—Dose-response curves for the depolarizing actions of acetylcholine and carbachol on isolated rat ganglia were established before and after exposure for 1 hr to physostigmine (10^{-5} g/ml.) or for 2 hr to dyflos (10^{-5} g/ml.). Preliminary experiments indicated that these concentrations of the two anticholinesterase agents potentiated maximally the effect of acetylcholine on normal ganglia. From the dose-response curves the ED50 values before and after anticholinesterase treatment were obtained, and the degree of potentiation calculated (Table 2).

Physostigmine increased the sensitivity of the normal rat ganglion to acetylcholine by about 100 times. It also shortened the time-course and increased the magnitude of the after-hyperpolarization, as shown in Fig. 2. Dyflos appeared to produce a larger increase in the sensitivity of the ganglion to acetylcholine than did physostigmine. Its effect on the after-hyperpolarization was similar to that of physostigmine. The effects of physostigmine and dyflos on the responses of denervated ganglia to acetylcholine were closely similar to those seen with normal ganglia.

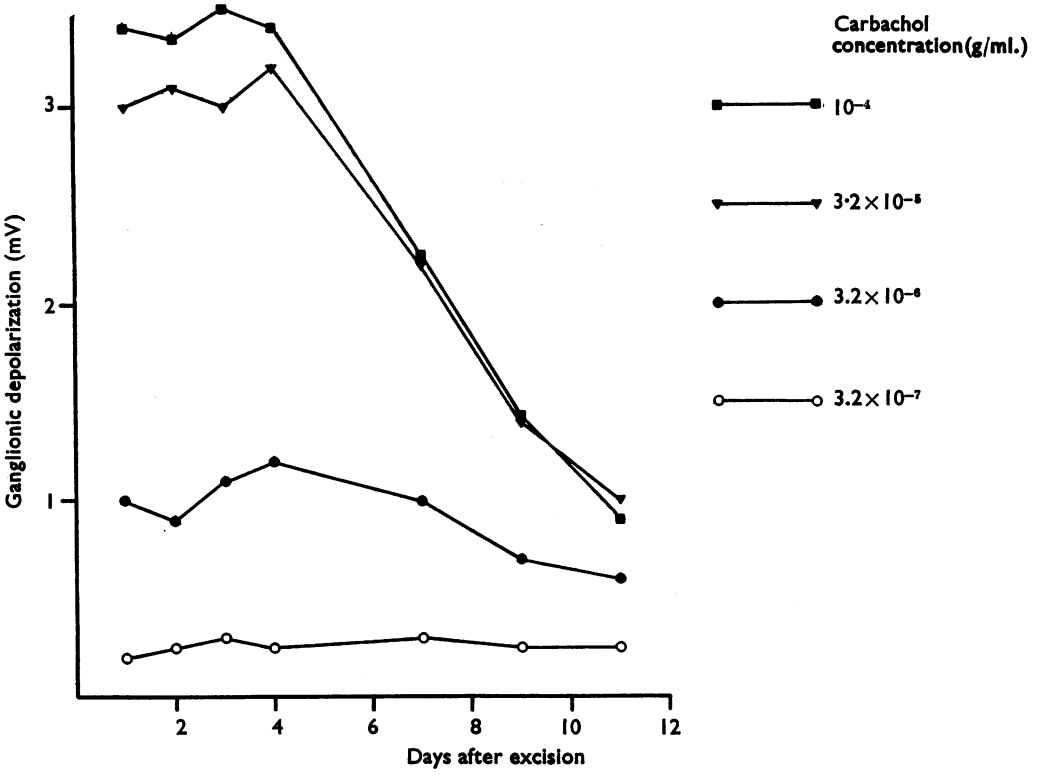

Fig. 4. Depolarization of an isolated rat superior cervical ganglion by carbachol at different times after excision of the ganglion from the rat. Ordinate, ganglionic depolarization (mV). Abscissa, time after excision (days).

TABLE 2

EFFECTS OF ANTICHOLINESTERASE AGENTS ON THE DEPOLARIZING ACTIONS OF ACETYLCHOLINE AND CARBACHOL ON THE ISOLATED RAT SUPERIOR CERVICAL GANGLION

(Each value refers to a single experiment)

Depolarizing agent	Anticholinesterase agent	Normal ganglia: ED50, g/ml. Control	Normal ganglia: ED50, g/ml. + anti-ChE	Normal ganglia: Potentiation (+) Inhibition (−)	Denervated ganglia: ED50, g/ml. Control	Denervated ganglia: ED50, g/ml. + anti-ChE	Denervated ganglia: Potentiation (+) Inhibition (−)
Acetylcholine	Physostigmine, 10^{-5}	$1{\cdot}2\times10^{-4}$	$1{\cdot}2\times10^{-6}$	+100	$1{\cdot}5\times10^{-4}$	$1{\cdot}9\times10^{-6}$	+ 79
		$1{\cdot}0\times10^{-4}$	$1{\cdot}3\times10^{-6}$	+ 77	$1{\cdot}2\times10^{-4}$	$1{\cdot}4\times10^{-6}$	+ 86
		$1{\cdot}0\times10^{-4}$	$2{\cdot}0\times10^{-6}$	+ 50			
		$1{\cdot}4\times10^{-4}$	$0{\cdot}72\times10^{-6}$	+190			
	Dyflos, 10^{-5}	$2{\cdot}1\times10^{-4}$	$1{\cdot}8\times10^{-6}$	+117	$1{\cdot}5\times10^{-4}$	$0{\cdot}58\times10^{-6}$	+270
		$1{\cdot}0\times10^{-4}$	$0{\cdot}53\times10^{-6}$	+190	$0{\cdot}92\times10^{-4}$	$0{\cdot}59\times10^{-6}$	+150
Carbachol	Physostigmine, 10^{-5}	$3{\cdot}2\times10^{-6}$	$8{\cdot}5\times10^{-6}$	−2·7	$3{\cdot}6\times10^{-6}$	$4{\cdot}2\times10^{-6}$	−1·2
		$1{\cdot}6\times10^{-6}$	$6{\cdot}3\times10^{-6}$	−4·8	$5{\cdot}3\times10^{-6}$	$4{\cdot}3\times10^{-6}$	+1·2
	Dyflos, 10^{-5}	$5{\cdot}1\times10^{-6}$	$4{\cdot}9\times10^{-6}$	+1·1	$7{\cdot}9\times10^{-6}$	$4{\cdot}4\times10^{-6}$	+1·8

Anticholinesterase agents had little effect on the sensitivities of normal or denervated ganglia to carbachol. No substantial modification of the time-course of the potential changes evoked by carbachol was seen after treatment with anticholinesterase drugs (see Fig. 2).

Isolated kitten superior cervical ganglia

Depolarization of isolated kitten ganglia by carbachol was much slower than that seen with rat ganglia: exposure of kitten ganglia to carbachol for up to 5 or 10 min was necessary before the depolarization reached a plateau. After-hyperpolarization was small and frequently absent. These differences from rat ganglion responses probably reflect the larger size and greater connective tissue content of the kitten ganglion, with consequent slower inward and outward diffusion of drug.

The maximal depolarization seen with kitten ganglia was less than that obtained with rat ganglia, and the sensitivity to carbachol measured by the ED50 (Table 1) only about half that of rat ganglia. These differences recall those between rabbit and rat ganglia described by Pascoe (1956), which were ascribed to the greater thickness and lower impedance of the postganglionic trunk in the rabbit when compared with the rat.

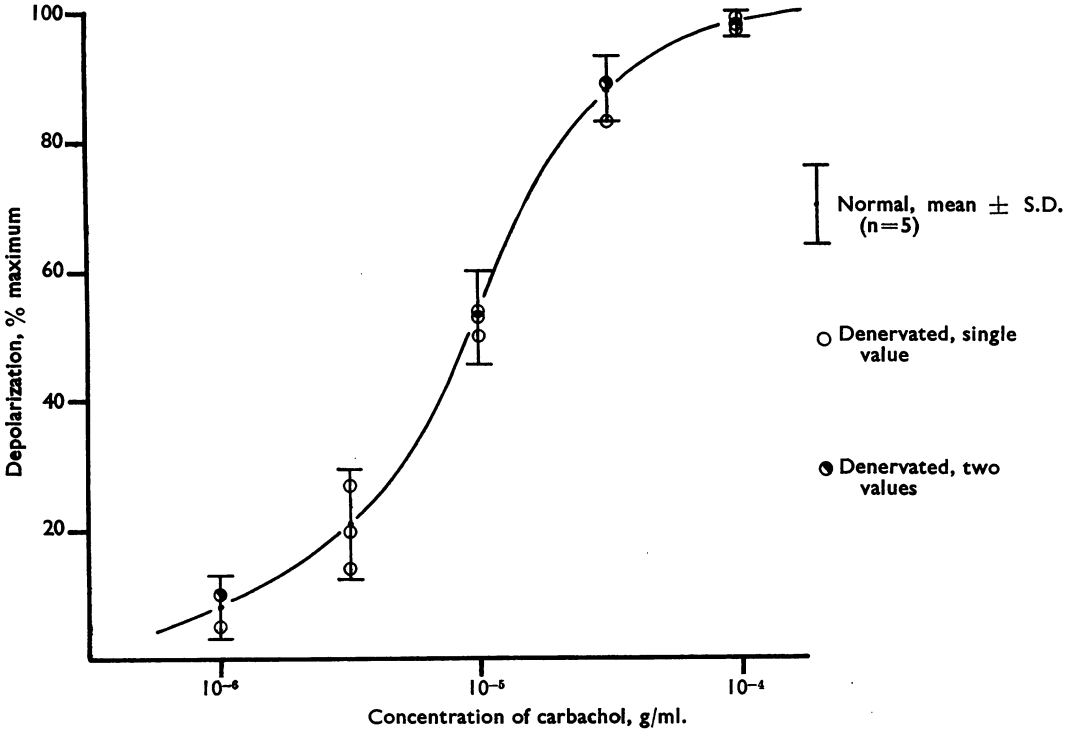

Fig. 5. Mean dose response curve for the depolarizing action of carbachol on isolated kitten superior cervical ganglia. Ordinate, ganglionic depolarization expressed as % of the maximal depolarization obtained. Abscissa, concentration of carbachol (g/ml.). The curve was drawn through the means of the values obtained using normal ganglia. Individual values obtained with preganglionically denervated ganglia are superimposed.

This explanation might equally well apply to the kitten ganglion, and would result in greater electronic spread of the ganglionic depolarization to the electrode leading from the postganglionic nerve trunk.

As with the rat ganglion, the dose-response curves for the depolarizing action of carbachol in normal and denervated kitten ganglia were closely similar (Fig. 5), and the sensitivities of normal and denervated ganglia measured by the ED50 values were not significantly different (Table 1). Attempts to measure the threshold depolarizing concentration of carbachol in kitten ganglia were thwarted by the very slow response, and by the occurrence of a small (50 to 150 μV) positive potential change of the ganglion relative to the postganglionic trunk (apparent ganglionic hyperpolarization) with concentrations of carbachol less than 10^{-6} g /ml.

DISCUSSION

Depolarization of isolated rat or kitten superior cervical ganglia by carbachol was not clearly altered following degenerative section of the preganglionic nerve trunk. This accords with the previous report (Volle and Koelle, 1961) that sympathetic ganglion cells do not show " denervation supersensitivity " to cholinesterase-resistant drugs.

Preganglionic denervation also did not affect the depolarizing action of acetylcholine on isolated rat superior cervical ganglia. This confirms the impression gained by Pascoe (1956) and Mason (1962). It suggests that the effective barrier to externally applied acetylcholine is provided by the cholinesterase of the capsular or glial cells, rather than by the cholinesterase of the preganglionic fibres, since the former, but not the latter, is unaltered following preganglionic denervation (Koelle, 1951 and 1955). The observation that the degree of potentiation of the depolarizing action of acetylcholine by physostigmine and dyflos was similar in normal and denervated ganglia accords with this view.

Measurement of postganglionic discharge in cats *in vivo* (Volle and Koelle, 1961) suggested that the superior cervical ganglion became less sensitive to carbachol after denervation. No comparable reduction in the depolarization of isolated ganglia by carbachol has been detected. It is not certain whether this discrepancy is indicative of a true difference between the effects of denervation on the two stages of response (depolarization and discharge) to drugs, or whether it is a reflexion of differences in technique, in particular between the modes of administration of drug. Investigation of the effect of denervation on the depolarization produced by drugs *in vivo* might help to resolve this uncertainty.

SUMMARY

1. Depolarization of isolated rat and kitten superior cervical ganglia by acetylcholine and carbachol was recorded with an external moving-fluid electrode system. Responses of normal and preganglionically denervated ganglia were compared.

2. With isolated rat ganglia the mean ED50 depolarizing concentrations (g/ml.) were: acetylcholine, normal: 1.45×10^{-4} (S.E., $\pm 0.16 \times 10^{-4}$); acetylcholine, denervated: 1.3×10^{-4} ($\pm 0.12 \times 10^{-4}$); carbachol, normal: 5.2×10^{-6} ($\pm 0.6 \times 10^{-6}$); carbachol, denervated: 5.0×10^{-6} ($\pm 8 \times 10^{-6}$). Maximum depolarizations of normal and

denervated ganglia were of similar size (about 3 mV). "Threshold" concentrations of carbachol required to produce 50 μV depolarization were 9 and 18×10^{-9} g/ml. with 2 normal ganglia and 4 and 7×10^{-9} in 2 denervated ganglia.

3. With isolated kitten ganglia mean ED50 values for carbachol were: normal, 9.4×10^{-6} (S.E., $\pm 0.9 \times 10^{-6}$) g/ml.; denervated, 9.1×10^{-6} ($\pm 0.5 \times 10^{-6}$).

4. Anticholinesterase drugs (physostigmine, 10^{-5} g/ml.; dyflos, 10^{-5}) potentiated the depolarizing action of acetylcholine on normal and denervated rat ganglia by similar amounts, and modified the time-course of the responses. Anticholinesterase drugs did not modify the responses of either normal or denervated rat ganglia to carbachol.

5. It is concluded that the depolarization of isolated superior cervical ganglia by acetylcholine or carbachol is not substantially changed by degenerative section of the preganglionic nerve trunk, contrary to the "law of denervation supersensitivity."

I would like to thank Mr P. M. G. Bell for assistance with the electronic apparatus, and Dr D. F. J. Mason for advice and for help in denervation of ganglia. The sample of dyflos used was kindly donated by the Chemical Defence Establishment at Porton Down.

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