RESEARCH PAPERS

CURARE-LIKE DRUGS AND VAGAL SYNAPSES: COMPARATIVE STUDY *IN VITRO* ON THE ISOLATED VAGUS-STOMACH PREPARATION OF THE RAT

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Received April 16, 1962

The anti-acetylcholine properties of curare-like drugs have been investigated on the motor responses of the electrically stimulated isolated vagus-stomach preparation of the rat. The synaptic site of the inhibitory action shown to varying degrees by the compounds tested has been located by comparing the modifications of the gastric responses to stimulation of preganglionic vagal fibres with the unmodified responses of the rat stomach strip preparation to acetylcholine.

PREVIOUS work on a series of curare-like drugs has uncovered a point of very considerable interest (Della Bella, Rognoni and Gopal, 1961). Much better correlation was found between *in vivo* neuromuscular blocking action than ganglion blocking action and the ability of these drugs to block the vagal ganglia in isolated vagus-heart preparations made from guinea-pigs. We have continued this investigation to determine whether this same correlation holds between *in vivo* neuromuscular blocking action and ability to block vagal ganglia at another site. We have chosen the rat stomach for this purpose and have used the intact stomach with vagi attached, prepared as recently described by Della Bella and Rognoni (1961). A parallel series of observations were made on strips of rat stomach stimulated by the addition of acetylcholine to the organ bath. The curare-like drugs examined were: (+)-tubocurarine, decamethonium, suxamethonium, succinyldisulphocholine, gallamine, hexafluorenium, laudexium and hexacarbacholine.

EXPERIMENTAL

Methods

White rats weighing 200 to 250 g. were used. The vagus-stomach preparation was made according to the method recently developed by Della Bella and Rognoni (1961). The whole stomach, isolated with vagus nerves intact, was suspended in a 100 ml. bath filled with oxygenated Ringer's solution at 30°. The gastric cavity, distended with 8 to 12 ml. of Ringer's fluid, was connected to a recording system consisting of a Marey tambour which, in turn, was connected to an isotonic lever having stops above and below. Thus it was possible to record the suitably amplified pressure variations occurring in the gastric cavity resulting from the muscle contractions caused by electrical stimulation of preganglionic vagal fibres. Stimulation was effected by means of platinum electrodes immersed in the bath. Rectangular pulses of 1 msec. duration

were applied at a frequency of 1–5/sec. in an alternating pattern of 3 min. of stimulation and 3 min. of rest. The solutions of the drugs to be tested were added directly to the organ bath 2 min. before electrical stimulation; the drug was removed by prolonged washing of the preparation.

The stomach strip preparation was made according to the method described by Vane (Vane, 1957): the strip was suspended in a 20 ml. bath kept at a temperature of 32° and connected to an isotonic lever, tension 2 g., with a 20-fold amplification of the contraction. Acetylcholine was used as a stimulant.

RESULTS

Experiments on the isolated vagus-stomach preparation of the rat have confirmed previous results obtained on cardiovagal synapses, showing clearly a marked anti-vagal property of some curare-like drugs. The results of a quantitative evaluation of the extent of inhibition exerted on the gastric motor responses to electrical stimulation are shown in Table I.

TABLE I

RELATIVE INHIBITORY POTENCY OF CURARE-LIKE DRUGS AND OF HEXAMETHONIUM ON THE RESPONSES OF THE ISOLATED VAGUS-STOMACH PREPARATION OF THE RAT TO ELECTRICAL STIMULATION

Drug	ED50 μg./ml.	Equipotent molar ratio (hexamethonium = 1·00)
Hexamethonium iodide	2·4	1
Hexafluorenium bromide	1	0·27
Gallamine iodide	10	2·1
Laudexium methylsulphate	5	0·9
Decamethonium iodide	12	3·9
Tubocurarine chloride	18	4·3
Hexacarbacholine bromide	20	7
Suxamethonium chloride	>500	>26·5
Succinyldisulphocholine iodide	>500	>17

Thus, the vagal blocking action of hexafluorenium appears to be approximately three times that of hexamethonium (Fig. 1). Also laudexium, the ganglion blocking action of which on the isolated guinea-pig intestine stimulated with nicotine was demonstrated by Collier and Macauley (1952), was found slightly more potent than hexamethonium. However, in contrast to observations made on the intestine, where laudexium has one-third to one-fourth the activity of tubocurarine, the relationship is reversed on the vagus-stomach preparation. Indeed laudexium proved approximately four times as potent as tubocurarine and even slightly superior to hexamethonium. Fig. 2 shows also that the inhibitory effect of laudexium is much more rapidly reversible than that of tubocurarine. Decamethonium was found virtually as active as tubocurarine and gallamine had even slightly greater activity. An estimate of the relative potency of the latter drug and hexamethonium is shown in Fig. 3. Hexacarbacholine showed only slight vagal blocking action. Suxamethonium and its disulphonium analogue proved even less active. Concentrations of the last three compounds somewhat lower

than those required markedly to antagonise responses to vagal stimulation, caused the appearance of a direct motor response of the organ, probably related to the known nicotine-like property of the two compounds (Bovet, Bovet-Nitti, Guarino, Longo and Marotta, 1949; Della Bella, Villani

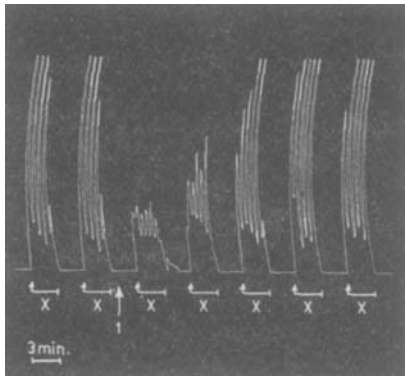

FIG. 1. The gastric motor responses after electrical stimulation of the vagal nervous supply. At X: electrical stimulation for 3 min. (frequency: 5 pulses/sec.; pulse duration: 1 msec.). The preparation was washed at the end of the stimulation period which was repeated at 3 min. intervals. At 1: hexafluorenium (1·5 μg./ml.) 2 min. before electrical stimulation.

and Zuanazzi, 1956); sometimes appreciable enhancement of the responses to electrical stimulation was also observed. Fig. 4 indicates one of the experiments effected with suxamethonium. In further experiments conducted on strips of rat stomach stimulated by acetylcholine, we investigated the influence exerted by the drugs tested on the effector structures.

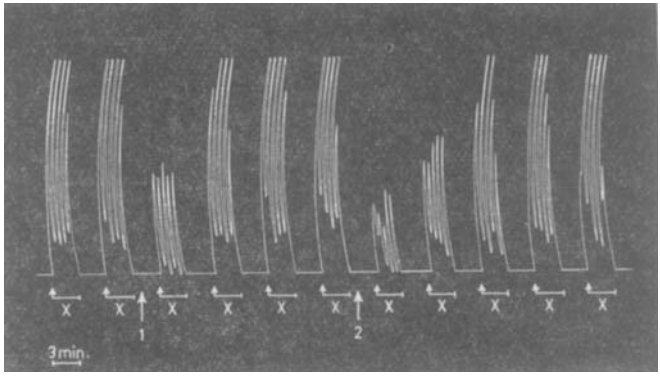

FIG. 2. The gastric motor responses after electrical stimulation of the vagal nervous supply. At X: electrical stimulation for 3 min. (frequency: 5 pulses/sec.; pulse duration: 1 msec.). The preparation was washed at the end of the stimulation period which was repeated at 3 min. intervals. Modification of the responses due to laudexium (5 μg./ml.) (at 1) and to tubocurarine (20 μg./ml.) (at 2) added to the bath 2 min. before electrical stimulation.

Since it is well known that curare-like drugs do not interfere with the liberation of the cholinergic mediator, the results of the above experiments should enable us to locate their site of action, in inhibiting the gastric motor responses to electrical stimulation of the preganglionic vagal fibres.

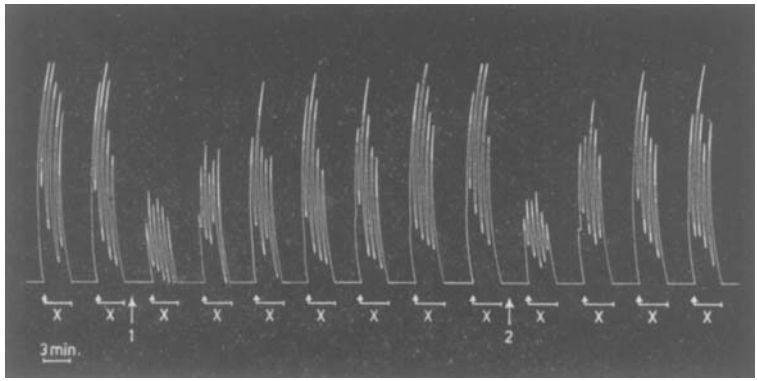

FIG. 3. The gastric motor responses after electrical stimulation of the vagal nervous supply. At X: electrical stimulation for 3 min. (frequency: 5 pulses/sec.; pulse duration: 1 msec.). The preparation was washed at the end of the stimulation period which was repeated at 3 min. intervals. Modifications of the responses due to hexamethonium (2·5 μg./ml.) (at 1) and to gallamine (10 μg./ml.) (at 2) added to the bath 2 min. before electrical stimulation.

It is of interest to describe the behaviour of stomach strips to various drugs: while no direct stimulant action was observed for tubocurarine and gallamine, this was particularly evident for decamethonium, hexafluorenium and for suxamethonium; the stimulation caused by laudexium and hexacarbacholine was of no constant pattern.

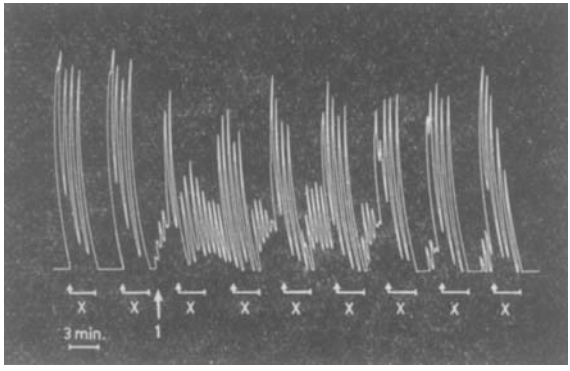

FIG. 4. The gastric motor responses after electrical stimulation of the vagal nervous supply. At X: electrical stimulation for 3 min. (frequency: 5 pulses/sec.; pulse duration: 1 msec.). The preparation was washed at the end of the stimulation period which was repeated at 3 min. intervals. Nicotine-like effect and modifications of the responses due to suxamethonium (0·35 mg./ml.) (at 1) added to the bath 2 min. before electrical stimulation.

As shown in Figs. 5 and 6, none of the compounds examined caused reduction of the gastric motor responses to the stimulation by acetylcholine.

Moreover, while decamethonium did not interfere with responses of the organ to acetylcholine, these were found markedly increased after a pretreatment with hexafluorenium. This sensitisation to acetylcholine might be attributed to the anticholinesterase activity shown by hexafluorenium both *in vitro* and *in vivo* (Rizzi, 1957; Della Bella, Rognoni and Gopal, 1961).

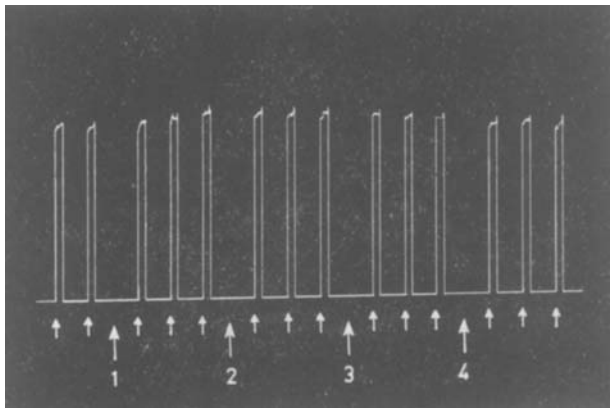

FIG. 5. Rat stomach strip preparation. At the arrows: the preparation is stimulated by adding to the bath acetylcholine of 0·05 μg./ml. (after 30 sec. contact, prolonged washing out). At 1: tubocurarine (25 μg./ml.) added to the bath, 1 min. before acetylcholine. At 2: gallamine (20 μg./ml.) added 1 min. before acetylcholine. At 3: laudexium (5 μg./ml.) added 1 min. before acetylcholine. At 4: hexacarbacholine (15 μg./ml.) added 1 min. before acetylcholine.

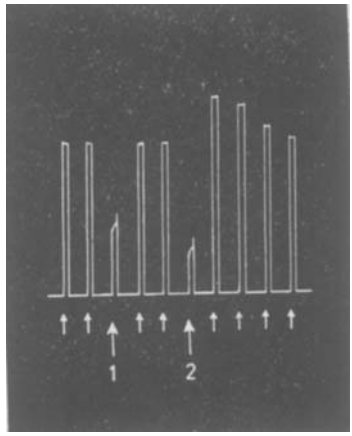

FIG. 6. Rat stomach strip preparation. At the arrows: the preparation is stimulated by adding to the bath acetylcholine (0·05 μg./ml.) (after 30 sec. contact, prolonged washing out). At 1 and 2: effect of direct stimulation by decamethonium (10 μg./ml.) (1) and by hexafluorenium (5 μg./ml.) (2) (after 30 sec. contact, prolonged washing out).

Discussion

The results described above clearly show the ability of the curare-like drugs tested to antagonise cholinergic transmission in vagal ganglia. The inhibitory effects produced by the compounds on the electrically driven stomach preparation were similar to those obtained at the cardiovagat synapses and, as observed in this latter case, bear little or no relation to their ganglion blocking activity *in vivo* (Della Bella, Rognoni and Gopal, 1961). The ganglionic site of action of the effects studied was evidenced, indirectly, by the experiments showing that the responses of the stomach strip to acetylcholine treatment were not modified by the same compounds.

Now, although both experimental and clinical evidence of the influence of curare-like drugs on gastrointestinal motility is scanty and does not cover all the compounds examined: tubocurarine (Gross and Cullen, 1945), gallamine (Riker and Wescoe, 1951), laudexium (Collier and Macauley, 1952), nevertheless, the existence of a marked behavioural difference between the activity displayed *in vivo* and that *in vitro* cannot be doubted. As previously reported (Della Bella, Rognoni and Gopal, 1961) it is not possible at the present moment to give an adequate explanation for these differences, but a working hypothesis would appear the one advanced by Cavallito, suggesting that the selectivity of action exhibited by the above drugs might be due to their different physicochemical properties. On these properties, in fact, depend both the distribution of the drugs in the body and the possibility and ease of their reaching various sites of action (Cavallito and Gray, 1960).

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