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The interaction of hemicholinium-3 and oxotremorine in isolated organ preparations

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On isolated ileum preparations of the rat and guinea-pig, hemicholinium-3 antagonizes contractions elicited by acetylcholine and oxotremorine to the same extent. Hemicholinium-3 was a mild antagonist to acetylcholine but a stronger one to oxotremorine and carbachol on the ileum of the rabbit. Whereas hemicholinium-3 has no anti-acetylcholine activity on the isolated urinary bladder of the rat, it antagonizes the contractions elicited by oxotremorine and carbachol, and acetylcholine after eserine. Morphine has an anti-oxotremorine activity on this organ. Increasing concentrations of oxotremorine release increasing amounts of acetylcholine from the rat isolated intestine.

The peripheral cholinergic action of oxotremorine is generally thought to be of direct postsynaptic origin because cholinesterase-blocking agents do not aggravate the fall in blood pressure it produces (Cho, Haslett & Jenden, 1962; Haslett, 1963) and because its spasmogenic effect on the guinea-pig intestine cannot be reduced with morphine, which is known to inhibit the release of acetylcholine (Lévy & Michel-Ber, 1967a). Lévy & Michel-Ber have also shown that eserine potentiates the action of oxotremorine on striated muscle preparations like the leech dorsal muscle and rat diaphragm. Paton & Aboo Zar (1968) report a possible action of tremorine on the nerve plexus.

Doubt has lately been cast on the direct cholinergic action of oxotremorine by the finding that the compound raises the level of acetylcholine in the brain of the rat (Holmstedt, Lundgren & others, 1965; Holmstedt, 1967) and the mouse (Lévy & Michel-Ber, 1967b). The tremor response to tremorine is depressed by compounds that inhibit acetylcholine synthesis, like hemicholinium-3 in chicks (Bowman & Osuide, 1968) and triethylcholine in rats (Slater & Rogers, 1968). This would appear to argue in favour of an indirect mechanism of action in the central nervous system both for oxotremorine and tremorine.

The present experiments were designed to find if the peripheral effects of oxotremorine could be influenced with hemicholinium-3. For this, a preparation was required in which the atropine-like activity of hemicholinium-3, observed by Bieger Lüllmann & Wassermann (1968) in the isolated atrium of the guinea-pig, would not be present. The urinary bladder of the rat proved satisfactory.

EXPERIMENTAL

The ileum from rats, guinea-pigs and rabbits, and the urinary bladder from rats were used. Contractions were recorded kymographically using an isotonic lever. The temperature of the oxygenated solution was maintained at 37° for the ileum and at 34° for the bladder. The composition of the solution used for all preparations was:

NaCl, 7·5; KCl, 0·41; $CaCl_2$, 0·24; $NaHCO_3$, 0·24; NaH_2PO_4, 0·14; glucose 1·0 g and distilled water 1000 ml. Contact time for the spasmogenic agents (acetylcholine oxotremorine and carbachol) was 0·5 min with ileum preparations and 1–2 min with bladders; the agents were always applied 15 min after hemicholinium-3. In analysing cumulative dose-effect relations on the rat isolated bladder the end-concentrations of the spasmogenic compounds were usually doubled at 1·5 min intervals.

To examine the acetylcholine-releasing action of oxotremorine, the small intestine, without the duodenum, of rats fasted for 24 h was cut into 9 pieces of roughly equal length, of which the 1st, 4th and 7th piece, the 2nd, 5th, and 8th piece, and the 3rd, 6th, and 9th piece were placed in separate vessels; in this manner each vessel contained 1·5–2·0 g of gut. In 8 ml of a solution containing eserine 10 μg/ml and gassed with O_2 at 37°, the pieces of gut were incubated for 10 min in the presence or absence of oxtremorine 5, 10 or 20 μg/ml. After 1 : 1·15 dilution with distilled water, the amount of acetylcholine in the incubation medium was determined on frog rectus abdominis muscle suspended in Ringer solution containing eserine (10 μg/ml). To identify acetylcholine as the agent producing the contractions the extracts were boiled in an alkaline medium or the agent was antagonized by tubocurarine. Oxotremorine itself produces no contractions even at concentrations as high as 20–30 μg/ml.

RESULTS

Rat isolated ileum. Hemicholinium-3, when used in doses of 30–300 μg/ml, antagonized acetylcholine- and oxotremorine-elicited contractions to the same extent.

Guinea-pig isolated ileum. Results similar to those with rat ileum were obtained: hemicholinium-3 (100 μg/ml) prevented the action of both acetylcholine and oxotremorine to approximately the same extent.

Rat isolated urinary bladder. Hemicholinium-3 (30 μg/ml given 1 to 3 times) usually did not affect acetylcholine-induced contractions but sometimes increased them. In contrast, hemicholinium-3 reduced the effects of oxotremorine and carbachol by 60 to 70% (Fig. 1). At higher concentrations, 60–200 μg/ml, hemicholinium-3 antagonized acetylcholine contractions 10–20%, whereas those caused by oxotremorine or carbachol were reduced 60–100%.

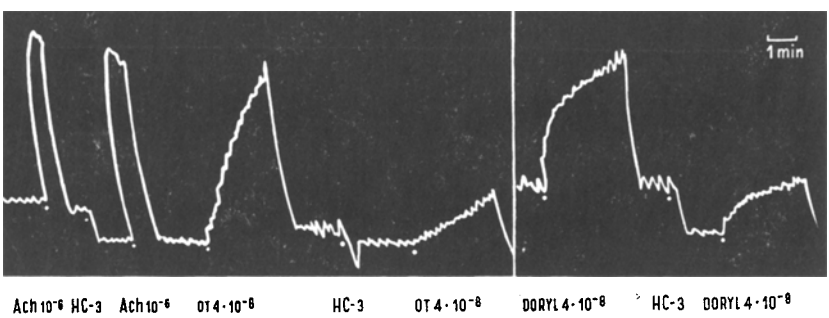

FIG. 1. Contractions of a rat urinary bladder, g/ml concentrations. HC-3: 3×10^{-5} g/ml hemicholinium-3 (Doryl = carbachol). OT = oxotremorine. Hemicholinium-3 reduces the effects of oxotremorine and carbachol by 60–70%.

In the presence of eserine (1 μg/ml), low concentrations of hemicholinium-3 (30 μg/ml) inhibited the action of acetylcholine to the same extent as it did that of the other two spasmogenic substances (Fig. 2).

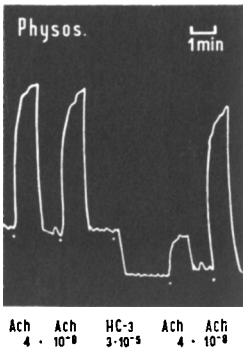

FIG. 2. Contractions of a rat urinary bladder in the presence of 10^{-6} g/ml eserine. A low concentration of hemicholinium-3 now inhibits acetylcholine action to the same extent as the other spasmogens. Concentrations in g/ml.

At 10 μg/ml hemicholinium-3 was inactive, whilst at 30 μg/ml it produced a shift to the right of the dose-response curve for oxotremorine but the maximum remained unaffected (Fig. 3), a similar shift was observed with carbachol, and also with acetylcholine, but eserine was then required. The antagonistic effects against each of the three compounds was nearly identical: the pA_2 mean values of 4 experiments was 4·59 $\pm$ 0·41 for oxotremorine, 4·58 $\pm$ 0·29 for carbachol, and 4·695 $\pm$ 0·31 for acetylcholine ($\pm$ values: probability intervals, P 95).

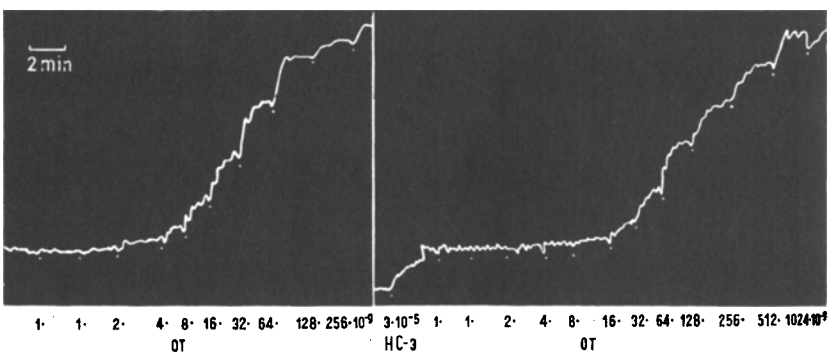

FIG. 3. Registrogram of cumulative dose-response curves obtained with oxotremorine (OT, $n \times 10^{-9}$ g/ml) on the isolated urinary bladder of the rat before and after hemicholinium-3 (HC-3, 3×10^{-5} g/ml).

In ten experiments with the bladder it was found invariably that morphine (50 μg/ml) increased the magnitude of acetylcholine-induced contractions by about 50% but reduced oxotremorine-elicited contractions by some 25%. Eserine 0·1 μg/ml increased the effect of low oxotremorine concentrations 1·5–2·5 fold.

Rabbit isolated ileum. At 30 μg/ml, hemicholinium-3 had no effect on acetylcholine-induced contractions but it reduced by about 30% those elicited by oxotremorine. At 100–300 μg/ml, hemicholinium-3 was a mild antagonist to acetylcholine (up to 23%) and a strong antagonist to oxotremorine and carbachol (40 to 80%) (Fig. 4).

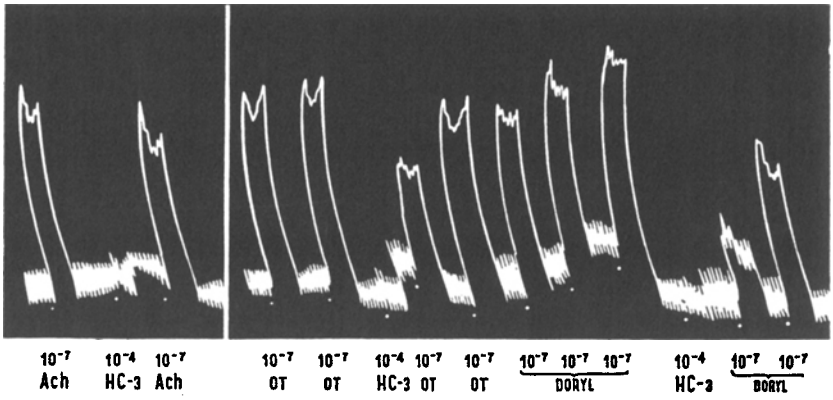

FIG. 4. Contractions of a rabbit ileum, g/ml concentrations (Doryl = carbachol). At 3×10^{-5} g/ml, hemicholinium-3 had no effect on acetylcholine-induced contractions but it reduced by about 30% those elicited by oxotremorine. At 10^{-4} g/ml, hemicholinium-3 was a mild antagonist to acetylcholine (up to 23%) and a strong antagonist to oxotremorine and carbachol (40 to 80%).

Acetylcholine release from rat gut. In only two of our experiments was acetylcholine released in appreciable amounts from rat isolated gut incubated in the absence of oxotremorine (Table 1). In the presence of oxotremorine, however, there was release, the rate of which was concentration dependent, doubling the oxotremorine concentration increasing the rate 1·3 to 3 fold. Boiling in alkaline medium or application of tubocurarine arrested activity in each case.

Table 1. *The effect of oxotremorine on the release of acetycholine from the isolated rat intestine, in ug/g of tissue.*

	Oxotremorine (μg/ml)			
	0	5	10	20
Acetylcholine μg/g	0·004–0·04	0·008–0·15	0·003–0·33	0·005–0·37
x/n	2/14	3/4	12/14	8/8

n = number of determinations x = number giving measurable release

DISCUSSION

Because of the atropine-like effect which hemicholinium-3 exerts on the small intestine of the rat and the guinea-pig, these preparations cannot be used to examine the "indirect cholinergic" action of oxotremorine. As the atropine-like activity of hemicholinium-3 is weak on the rabbit intestine, this preparation is more suitable for this purpose. But the rat isolated bladder is most appropriate because hemicholinium-3 has no anti-acetylcholine activity on it. It also has no parasympathetic ganglia, so is unaffected by ganglion stimulants (Hukovič, Rand & Vanov, 1965).

Our results with rat bladder preparations and our incubation experiments have provided evidence that both oxotremorine and carbachol possess properties that can be inhibited peripherally by hemicholinium-3, i.e. the release or activation of acetylcholine. The indirect, acetylcholine-releasing action of carbachol has been described by McKinstry & Koelle (1967) in sympathetic ganglia.

But hemicholinium-3 inhibits acetylcholine only in the presence of eserine, while markedly depressing carbachol-elicited contractions in its absence. We incline to the view that the immediate and short-acting action of acetylcholine destroyed by cholinesterase is a postsynaptic action whereas the lasting action of carbachol or that of acetylcholine after a cholinesterase inhibitor mobilizes or activates presynaptic acetylcholine—a view based on an earlier assumption of Koelle (1961; 1962).

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