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Actions of the muscle relaxant chandonium iodide on guinea-pig ileum and vas deferens preparations†

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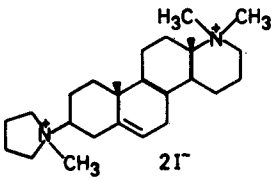
The effects of the newly synthesized neuromuscular blocking agent, chandonium iodide (17a-methyl-3β-pyrrolidino-17a-aza-D-homo-5-androstene dimethiodide) have been investigated on guinea-pig isolated ileum and vas deferens preparations. On the ileum, chandonium ($0{\cdot}1$–$10{\cdot}0\ \mu g\ ml^{-1}$; $1{\cdot}6 \times 10^{-7}$ M–$1{\cdot}6 \times 10^{-5}$ M) had weak muscarinic receptor blocking action (pA_2 is 5·7), but no antihistamine properties at the concentration tested. No evidence for anticholinesterase actions was found. On the vas deferens, chandonium (10–50 $\mu g\ ml^{-1}$; $1{\cdot}6$–$8{\cdot}1 \times 10^{-5}$ M) potentiated responses to exogenous noradrenaline; responses to electrical stimulation were potentiated only in the presence of 50 $\mu g\ ml^{-1}$ chandonium. No adrenoceptor or adrenergic neuron blockade was found. The results provide evidence that chandonium acts selectively at acetylcholine receptors and that it is more active as a nicotinic receptor antagonist than as a muscarinic receptor antagonist.

A series of aza-steroids in which one or both quaternary nitrogen groups are incorporated into the steroid nucleus has been developed (Singh, Paul & Parashar, 1972, 1973; Singh & Paul, 1974). Members of this series have been shown to antagonize the actions of acetylcholine at autonomic ganglia and neuromuscular junctions (Marshall, Paul & Singh, 1973 a & b; Gandiha, Marshall & others, 1974; 1975). Chandonium iodide (17a-methyl-3β-pyrrolidino-17a-aza-D-homo-5-androstene dimethiodide) is the most potent neuromuscular blocking agent in the series.



In the cat chandonium exhibits a potent, competitive, non-depolarizing neuromuscular blocking action that is rapid in onset and of short duration. It possesses a potent atropine-like action at the cardiac vagus neuroeffector junction but has little ganglion blocking activity. *In vitro* studies indicate that chandonium possesses weak anticholinesterase activity (Gandiha & others, 1975). It also antagonizes the response to acetylcholine in skeletal muscle grown in cell culture (Harvey, Paul & Singh, 1975).

† This is part XXXVII of Steroids and related studies.

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The specificity of chandonium has been further investigated using guinea-pig isolated ileum and vas deferens preparations. Some of these results were presented to the Indian Pharmaceutical Congress, Patna December, 1975 (Harvey, Paul & others, 1976).

METHODS

Guinea-pig ileum preparation

Segments of ileum 1–2 cm in length were suspended with a resting tension of approximately 0·5 g in oxygenated Tyrode solution (1910). The temperature was maintained constant during each experiment, within the range 31–34°. Agonists were added every 3 min with a contact time of 20 s. Responses were measured isometrically with Grass FTO3C force displacement transducers, and recorded on Grass 79B polygraphs.

Guinea-pig vas deferens preparation

Vasa deferentia 1–3 cm in length were suspended with a resting tension of approximately 0·5 g in oxygenated Tyrode solution. The temperature was maintained constant during each experiment, within the range 31–34°. Ring electrodes were placed around the lower portion of the preparations. Every 10 min the preparations were stimulated by 5 s trains of pulses (1 ms, 20Hz) at a voltage greater than that necessary to evoke maximal responses. Midway between each train of pulses, noradrenaline was added in concentrations sufficient to produce contractions similar in size to the responses elicited by electrical stimulation. The sensitivity to exogenous noradrenaline varied between preparations,

the range used being 2–60 μg ml^{-1}. Drug contact time was 30 s. Responses were measured isometrically.

Drugs

The drugs used were acetylcholine chloride, carbachol (carbamylcholine) chloride and noradrenaline hydrochloride (Sigma), histamine acid phosphate (British Drug Houses) and chandonium iodide. The concentrations quoted in the text refer to free base, except for chandonium iodide which is the salt. All drugs were freshly prepared in Tyrode solution.

Treatment of results

Statistical evaluation was performed using Student's *t*-test. Differences between the means were taken as significant when $P < 0.01$.

RESULTS

Guinea-pig ileum preparation

Control concentration-effect curves were obtained with acetylcholine and carbachol. The bathing solution was then changed to Tyrode containing chandonium. During a 30 min equilibration period alternate additions of acetylcholine and carbachol were made. After 30 min in chandonium, concentration-effect curves to the agonists were again obtained. The preparations were returned to normal Tyrode and after a similar 30 min equilibration period concentration-effect curves were established.

The effects of chandonium on the responses to acetylcholine and carbachol are shown in Figs 1 and 2, respectively. Above 1·0 μg ml^{-1} (1·6 × 10^{-6} M), chandonium produced concentration-dependent parallel shifts of the curves to the right. From plots

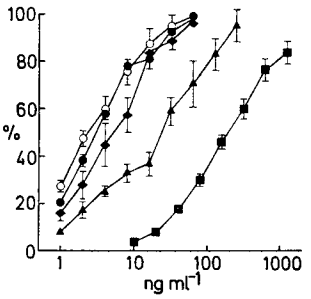

FIG. 1. Concentration-effect curves on isolated ileum preparations to acetylcholine in the absence of (●) and in the presence of 0·1 μg ml^{-1} (1·6 × 10^{-7} M) (○); 1·0 μg ml (1·6 × 10^{-6} M) (◆); 5·0 μg ml^{-1} (8·1 × 10^{-6} M) (▲); and 10·0 μg ml^{-1} (1·6 × 10^{-5} M) (■) chandonium. Each point represents the mean of at least 4 determinations; s.e. bars are shown when they are larger than the symbols. y axis-response (% maximal).

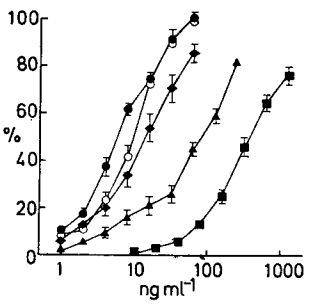

FIG. 2. Concentration-effect curves on isolated ileum preparations to carbachol in the absence of (●) and in the presence of 0·1 μg ml^{-1} (1·6 × 10^{-7} M) (○); 1·0 μg ml^{-1} (1·6 × 10^{-6} M) (◆); 5·0 μg ml^{-1} (8·1 × 10^{-6} M) (▲); and 10·0 μg ml^{-1} (1·6 × 10^{-5} M) (■) chandonium. y axis-response (% maximal).

of log (dose ratio-1) against pAx (Arunlakshana & Schild, 1959), the extrapolated values for the pA_2 of chandonium were 5·7 against acetylcholine and 5·8 against carbachol.

Although 30 min was allowed for equilibration in chandonium, the blockade developed rapidly in all preparations. The blocking effects of chandonium were easily reversed by washing. The concentration-effect curves obtained 30 min after washout had returned to control values.

The effects of chandonium on responses to histamine were tested using a similar procedure as for carbachol and acetylcholine. Chandonium at 10 μg ml^{-1} (1·6 × 10^{-5} M) did not change the concentration-effect curve to histamine.

Guinea-pig vas deferens preparation

The effects of chandonium (10 and 50 μg ml^{-1}; 1·6 × 10^{-5} M and 8·1 × 10^{-5} M) on electrical stimulation and added noradrenaline are shown in Fig. 3. At both concentrations, chandonium produced a significant increase in the responses to noradrenaline. In the presence of 10 μg ml^{-1} (1·6 × 10^{-5} M) chandonium the noradrenaline response was 146 ± 9% (mean ± s.e.) of the control values; at 50 μg ml^{-1} (8·1 × 10^{-5} M) chandonium the response was 180 ± 10·5% of the control values. The responses to electrical stimulation were also increased in the presence of chandonium but the increase was only significant with the higher concentration, when the response increased to 130 ± 6·5% of control.

At both concentrations, chandonium was rapid in onset of action and easily washed out. After 30 min of washing out chandonium, the responses to electrical stimulation and added noradrenaline were significantly reduced from the control values after 10 μg

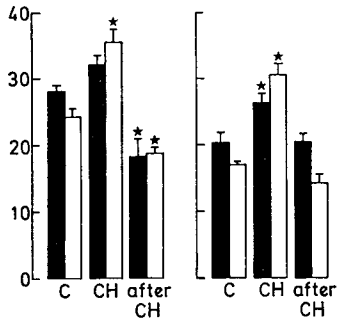

FIG. 3. Responses of isolated vas deferens preparations (mm) to electrical stimulation and to noradrenaline. The left hand panel illustrates the effects of 10 μg ml^{-1} ($1{\cdot}6 \times 10^{-5}$ M) chandonium, the right hand panel the effects of 50 μg ml^{-1} ($8{\cdot}1 \times 10^{-5}$ M) chandonium. Shaded columns are the responses to electrical stimulation; clear columns are the responses to added noradrenaline. Each column represents the mean response of at least 4 preparations; the bars indicate standard errors. * = significantly different from control values ($P < 0{\cdot}01$). C—control. CH—chandonium.

ml^{-1} ($1{\cdot}6 \times 10^{-5}$ M) chandonium but not after 50 μg ml^{-1} ($8{\cdot}1 \times 10^{-5}$ M) chandonium.

DISCUSSION

The experiments described establish that chandonium has weak muscarinic receptor blocking activity in guinea-pig ileum. This action would appear to be selective for the cholinoceptors since chandonium at the highest concentration used did not reduce responses to histamine.

The pA_2 values of chandonium at muscarinic receptors were 5·7 against acetylcholine and 5·8 against carbachol. Although chandonium is a weak anticholinesterase (Gandiha & others, 1975), the lack of difference in the pA_2 values against acetylcholine and the cholinesterase-stable agonist, carbachol, suggests that inhibition of acetylcholinesterase by chandonium did not influence its blocking action.

The prototype compound of the aza-steroid series, HS-342 (4,17a-dimethyl-4,17a-diazo-D-homo-5α-androstane dimethiodide), has a pA_2 of 5·2 at muscarinic receptors (Marshall & others, 1973a; Gandiha & others, 1974), and the pA_2 for atropine at muscarinic receptors is 8·4 (Schild, 1947). Therefore, chandonium and HS-342 are approximately equipotent in their blocking action at muscarinic receptors but they are about 1000 times less potent than atropine. Chandonium is much more active as an antagonist at nicotinic receptors; it has a pA_2 value of 8·1 against carbachol in the chick biventer cervicis muscle preparation (Gandiha & others, 1975). Corresponding values for HS-342 and tubocurarine in the same preparation are 6·4 and 6·0, respectively (Marshall & others, 1973a; Gandiha, Green & Marshall, 1972). Chandonium is, therefore, about 70 times more potent than HS-342 as a neuromuscular blocking agent.

In the vas deferens preparation chandonium did not have α-adrenoceptor or adrenergic neuron blocking actions. These results substantiate the *in vivo* findings of Gandiha & others (1975). The responses to electrical stimulation and to added noradrenaline were increased significantly in the presence of chandonium. This effect could be due to inhibition by chandonium of the uptake$_1$ inactivation process for noradrenaline.

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