THE EFFECTS ON ANIMALS OF LARGE DOSES OF LAUDEXIUM METHYLSULPHATE

BY

H. O. J. COLLIEN AND BARBARA MACAULEY

With an Appendix by LOIS H. BROWN

From the Research Division, Allen and Hanburys Ltd., Ware, Herts.

LAUDEXIUM METHYLSULPHATE (Laudolissin) is a synthetic curarizing agent (Taylor and Collier, 1951) used in prolonged surgical operations. We have previously described some of its pharmacological properties (Collier and Macauley, 1952) and the purpose of the present paper is to report a further exploration of its pharmacology in relation to its clinical use.

As Bovet (1951) has stressed, the toxicity of a neuromuscular blocking agent in animals unprovided with artificial respiration indicates its potency rather than its possible toxicity during anaesthesia. More information may be gained by assessing the toxicity of these agents in animals maintained by artificial respiration and we describe such an assessment of laudexium.

Since it seems possible that laudexium may be administered clinically over long periods of time to give muscular relaxation in chronic conditions, we also report results of sub-acute and chronic toxicity tests of the drug in mice.

MATERIALS AND METHODS

The methylsulphates of laudexium and neostigmine, the chlorides of d-tubocurarine, acetylcholine and suxamethonium, the hydrochlorides of papaverine and tetrahydropapaverine, the sulphate of atropine and the maleate of mepyramine were used. For brevity, drugs are referred to by the name of the base only, though weights given are those of the salts.

Most of the methods used have previously been described (Collier and Macauley, 1952), except the following. Salivation was recorded in anaesthetized cats by a Condon drop-recorder, connected with a metal cannula inserted in the submaxillary duct. Rabbits were anaesthetized with an intravenous dose of 0.75 mg. urethane per kg.

RESULTS

Sialogogic Action.

In 8 cats prepared for recording the flow of saliva, intravenous doses of up to 3 mg. laudexium per kg. failed to evoke a response, but larger doses sometimes produced a flow. This is illustrated in figure 1, in which experiment 4 mg. laudexium produced about the same amount of salivation as 40 μg. neostigmine per kg. It will be seen, however, that the latency between administration of laudexium and the beginning of salivation was about half that after neostigmine. The characteristic hypotensive action of large doses of laudexium, which we have previously reported, is to be seen in this tracing.
Lack of Anticholinesterase Activity.

The fact that d-tubocurarine (Paton and Zaimis, 1949) and other neuromuscular blocking agents possess some anticholinesterase activity prompted investigation of this property in laudexium. Zaimis (1953) has used the production of muscular fasciculations in the rat as a rapid test of anticholinesterase activity in vivo, if direct muscle stimulation can be excluded. Since laudexium exhibits low curarizing activity in this species, we applied this test. Intravenous administration of laudexium at the largest dose that did not curarize (1 mg. per kg.) failed to produce fasciculations, although small doses of neostigmine (50 μg. per kg.) readily produced the effect.

In vitro tests for anticholinesterase activity were carried out by Miss L. H. Brown and are reported in the Appendix to this paper. In these, solutions of laudexium failed to inhibit the cholinesterases of either human plasma or red cells.

Actions on Guinea-pig's Ileum.

Feldberg and Smith (1953) have reported that d-tubocurarine and other histamine liberators "when added to the bath in which guinea-pig's ileum is suspended, produce periods of increased rhythmicity and tone." Laudexium in high concentrations had a somewhat similar effect; which is illustrated in figure 2. Both mepyramine and atropine antagonized this effect. It can be seen from figure 2 that atropine also reduced the spontaneous contractility of the preparation though mepyramine did not. The effect of atropine persisted long after it was washed out of the bath.

Since laudexium antagonizes the nicotinic effects of acetylcholine at the motor
EFFECTS OF LARGE DOSES OF LAUDEXIUM METHYLSULPHATE

**Fig. 2**

Guinea-pig's ileum preparation in 20 ml. bath. Response to laudexium. Intervals between tracings A—D, 4 min. each; between E and F, 10 min.; F and G, 20 min.

A—2.5 mg. laudexium; B—2.5 mg. laudexium 30 sec. after 1 mg. mepyramine; C—no drug; D—2.5 mg. laudexium; E—2.5 mg. laudexium 30 sec. after 10 /μg. atropine; F—2.5 mg. laudexium; G—2.5 mg. laudexium.

end-plate and in autonomic ganglia, we also investigated whether it antagonized the muscarinic effects of acetylcholine on the guinea-pig's ileum. It will be seen from figure 3 that both laudexium and d-tubocurarine in high concentrations exert an atropine-like effect on this response.

Since laudexium is related chemically to papaverine, we also explored the possibility that it might antagonize the spasm of guinea-pig's ileum produced by barium chloride. It will be seen from figure 4 that in very high concentrations laudexium exhibits a similar activity to papaverine and to tetrahydropapaverine. Laudexium is markedly less active than papaverine and the effect was not seen at concentrations inhibiting the response to acetylcholine. At the high concentrations of laudexium required to relax the barium spasm, the effect may be masked by initiation of the rhythmic contractions described above.

**Toxicity to Animals Under Artificial Respiration.**

Rabbits anaesthetized with urethane were given intravenously large doses of laudexium and maintained by means of a respiration pump. The condition of the heart was observed by palpation at intervals for four hours after treatment with the curarizing agent, unless the animal died sooner. It will be seen from table I, which gives the results of 13 experiments, that

<table>
<thead>
<tr>
<th>I.V. dose in mg. per kg.</th>
<th>Proportion of rabbits surviving</th>
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<tbody>
<tr>
<td>50</td>
<td>5/5</td>
</tr>
<tr>
<td>75</td>
<td>1/3</td>
</tr>
<tr>
<td>100</td>
<td>3/5</td>
</tr>
</tbody>
</table>

TABLE I

Toxicity of large doses of laudexium in rabbits maintained by artificial respiration.
all of 5 rabbits tolerated for four hours a dose of 50 mg. per kg., which is about a thousand times the LD50 in animals unprovided with artificial respiration. It is impossible to say how much longer the survivors would have lived if the experiments had not been terminated; but the hearts of all survivors were beating strongly at the end. All animals that died did so within $2\frac{1}{2}$ hours of treatment.

Two similar experiments were performed in cats under chloralose anaesthesia. One animal receiving 50 mg. laudexium per kg. survived for the four-hour period of the experiment. The other cat, receiving 100 mg. per kg., died within $2\frac{1}{2}$ hours of treatment.

Toxicity to Mice.

In acute subcutaneous toxicity tests, each involving 40 mice, the LD50 of laudexium was found to be 2.95 mg. per kg. and of d-tubocurarine 0.63 mg. per kg.

In a sub-acute toxicity test 20 mice received subcutaneous doses of 1 mg. laudexium per kg. on the first, second, third, fourth, fifth, eighth, ninth, tenth and eleventh days of the experiment. None died and on the twelfth day we compared the paralysing activity of
intravenous doses of laudexium on these mice with that in untreated controls, using the rotating drum. At an intravenous dose of 200 µg. per kg., 1 of 10 mice in the treated group was paralysed and subsequently died and 1 of 10 controls was paralysed and recovered. At 350 µg. all of 10 of the treated mice were paralysed and 6 died, while 8 of 10 controls were paralysed and one died. There appeared therefore to be a slightly greater sensitiveness to laudexium in the group that had previously received 9 doses of the drug.

In a chronic subcutaneous toxicity test, groups of 10 mice were treated daily except on Saturdays and Sundays, for 8 weeks. One group received 1.0 mg. laudexium per kg., another 0.2 mg. d-tubocurarine and a third 10 ml. saline. The mice were weighed regularly and at the end of 6 and 8 weeks their bloods were examined. The results of these observations, expressed in table II, give no evidence of toxic effects of either drug on growth or blood picture. After the fourth treatment with laudexium, one mouse died; but this does not appear to be important, since no further death occurred during 7 weeks and since all of 20 mice survived the same treatment during the sub-acute test. Another mouse in the same batch from the breeder died on the same day.

The behaviour and movement of the mice were normal at the end of 8 weeks, when they were sacrificed. No macroscopic lesions were seen at the site of injection. Sections of livers, kidneys, spleens and hearts were examined by Dr. V. Udall who found no changes attributable to either drug.
TABLE II
Weights and blood findings in mice treated during eight weeks with 40 subcutaneous doses of 1.0 mg. laudexium or 0.2 mg. d-tubocurarine per kg. body weight

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean weight in g. of group at weeks</th>
<th>Mean blood findings at 8 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Laudexium</td>
<td>20.2</td>
<td>24.5</td>
</tr>
<tr>
<td>d-Tubocurarine</td>
<td>20.2</td>
<td>25.1</td>
</tr>
<tr>
<td>Normal saline</td>
<td>20.7</td>
<td>25.2</td>
</tr>
</tbody>
</table>

DISCUSSION

In contrast with decamethonium and suxamethonium and like d-tubocurarine, laudexium does not stimulate the frog's rectus abdominis, but antagonizes the stimulatory effect of acetylcholine and like-acting substances upon it (Chesher and Collier, 1954). The side-effects seen with laudexium are such as might be expected from this antagonism to acetylcholine and similarity to d-tubocurarine. It is not surprising if such a drug as laudexium also antagonizes the actions of acetylcholine on autonomic ganglia (Collier and Macauley, 1952) and upon smooth muscle. What is surprising, however, is the high degree of specificity that laudexium exhibits at the skeletal muscle end-plate, as compared with other sites of action. Although it might be expected that laudexium would also possess anticholinesterase activity, it was not possible to detect it. This finding is particularly interesting because Smith, Pelikan, Maramba and Unna (1953) have found that comparable bis-polymethylene quaternary ammonium salts, linked through carbon atoms of the isoquinolinium rings, exhibit high anticholinesterase activity.

The interpretation of the stimulating effects of laudexium on salivary flow in the cat and on the tone and rhythmicity of the guinea-pig's ileum presents difficulties. The failure to detect anticholinesterase action in human blood renders it most unlikely that these effects are due to such action. The facts that (i) laudexium is known to release histamine; (ii) mepyramine inhibits the stimulating effect of laudexium on the guinea-pig ileum without abolishing rhythmicity and (iii) histamine can cause salivation in the cat (Dale and Laidlaw, 1910; Gibbs and McClanahan, 1937) make it possible that the effects of laudexium under discussion may be due to histamine release. This explanation is the same as that proposed by Feldberg and Smith (1953) for the stimulating effect of d-tubocurarine on the guinea-pig's ileum. Whether or not this is the correct explanation of the effects under discussion, it is to be noted that they are only seen at concentrations higher than the largest likely to occur in the human body during the clinical use of laudexium.

Reports have now been published on the use of laudexium in more than 1,328 surgical operations (Binning, 1953; Bodman, Morton and Wylie, 1952; Dundee, Gray and Riding, 1954; Fuller and Harrison, 1953; Grant-Whyte, 1953; Lederman, 1953; Pitcher, 1953; Sara, Marshall and Balthasar, 1954). It is of interest to compare some aspects of the
clinical findings with the results of animal experiments.

In surgical operations initial doses of laudexium have generally fallen between 10 and 60 mg. per patient and subsequent doses have not exceeded half the initial dose. Single doses have therefore probably not exceeded 1 nor total doses 4 mg. per kg. Among more than 1,328 patients the reported incidence of side-effects is very low. We have administered laudexium in doses ranging from 50 μg. to 100 mg. per kg. to a total of 63 cats under chloralose anaesthesia. In the cat, in which the potency of laudexium approximates to that in man, a single dose of 1 mg. per kg. (about 5 times the fully effective dose) caused no obvious side-effects with the exception of occasional slight hypotension. At this dose, therefore, side-effects appear to be very inconspicuous both in the cat and in man. At single doses exceeding 2 mg. per kg. in the cat, the side-effects described in this or the previous paper are liable to be seen, but there has been no record of the use in man of such high doses, which do not appear to be necessary.

SUMMARY

(1) Very large doses of laudexium sometimes provoked slight salivation in the cat.

(2) Laudexium did not evoke muscular fasciculations in the rat at the largest intravenous dose (1 mg. per kg.) that did not curarize.

(3) Laudexium at a concentration of $M \times 10^{-8}$ in human red cells or plasma did not inhibit the cholinesterases in vitro. In parallel tests, neostigmine at $M \times 10^{-4}$ to 10$^{-8}$ inhibited the cholinesterases by 50 per cent (see appendix).

(4) High concentrations of laudexium increased the tone and rhythmicity of guinea-pig's ileum in vitro. Both effects were antagonized by atropine and the effect on tone by mepyramine.

(5) High concentrations of laudexium and of d-tubocurarine antagonized the response to acetylcholine of the guinea-pig's ileum in vitro. Still higher concentrations of laudexium relaxed the spasm caused by barium.

(6) Anaesthetized rabbits maintained by artificial respiration survived for four hours an intravenous dose of laudexium 1,000 times the LD50.

(7) All of 20 mice survived 9 subcutaneous doses of 1.0 mg. laudexium per kg. given over 11 days.

(8) Mice tolerated without effect on behaviour, growth, blood picture or microscopic appearance of certain organs 40 subcutaneous doses of 1.0 mg. laudexium or 0.2 mg. d-tubocurarine per kg. given over a period of 56 days.

ACKNOWLEDGMENTS

Our thanks are due to the following: Mr. N. E. Condon for the drop-recorder; Mr. G. K. Smith for blood counts and preparation of sections; Dr. V. Udall for examination of sections; the Directors of Allen and Hanburys Ltd., for permission to publish this paper.

REFERENCES


**APPENDIX**

**FAILURE TO DETECT ANTICHOLINESTERASE ACTIVITY IN LAUDEXIUM METHYLSULPHATE**

**BY**

**LOIS H. BROWN**

The determination of cholinesterase numbers (Ch. E. No.) by the modification of Michel’s electrometric method described by Aldridge and Davies (1952), using acetylcholine chloride as substrate, was employed in an attempt to detect anticholinesterase activity in laudexium methylsulphate (Laudolissin). As a standard for comparison neostigmine methylsulphate was used.

The cells and plasma of human blood were separated as described by Aldridge and Davies, the cells being used as a source of “true” and the plasma of “pseudo” cholinesterase. 0.1 ml. solutions of laudexium or neostigmine in normal saline

<table>
<thead>
<tr>
<th>Source of Cholinesterase</th>
<th>Test solution</th>
<th>Ch. E. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Expt. 1</td>
</tr>
<tr>
<td>Red cells</td>
<td>Control</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>Neostigmine,</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>$M \times 10^{-6}$</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>$M \times 10^{-8}$</td>
<td>70</td>
</tr>
<tr>
<td>Control</td>
<td>85</td>
<td>42.5</td>
</tr>
<tr>
<td>Laudexium</td>
<td>80</td>
<td>40</td>
</tr>
<tr>
<td>$M \times 10^{-3}$</td>
<td>80</td>
<td>37.5</td>
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<tr>
<td>$M \times 10^{-4}$</td>
<td>80</td>
<td>42.5</td>
</tr>
<tr>
<td>Plasma</td>
<td>Control</td>
<td>85</td>
</tr>
<tr>
<td>Neostigmine</td>
<td>70</td>
<td>90</td>
</tr>
<tr>
<td>$M \times 10^{-6}$</td>
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<td>10</td>
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<tr>
<td>$M \times 10^{-8}$</td>
<td>45</td>
<td>60</td>
</tr>
<tr>
<td>Control</td>
<td>107.5</td>
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<td>100</td>
<td>90</td>
</tr>
<tr>
<td>$M \times 10^{-3}$</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>$M \times 10^{-4}$</td>
<td>95</td>
<td>87.5</td>
</tr>
</tbody>
</table>
were added per 5 ml. cells or plasma. An equal volume of normal saline was added to the controls. The cholinesterase activities of these solutions were then estimated in the saponin-buffer mixture of Aldridge and Davies. Neostigmine exerted a 50 per cent inhibition at concentrations in cells or plasma between $M \times 10^{-6}$ and $M \times 10^{-4}$ but laudexium showed no activity at $M \times 10^{-3}$, which was the highest concentration it was possible to obtain by the method used. These results are expressed in the table. Control mixtures prepared exactly as above, but without addition of acetylcholine, showed no change of pH on incubation.

It was concluded from these experiments that laudexium methylsulphate at a concentration of $M \times 10^{-4}$ in human red cells or plasma did not inhibit the cholinesterases in vitro.

**ACKNOWLEDGMENT**

We are indebted to Roche Products Ltd., for the sample of neostigmine ("Prostigmin") methylsulphate.

**REFERENCE**


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**BOOK REVIEW**


This book contains a lot of useful practical information but is marred by fundamental errors and omissions and jargon has not been eliminated.

The first two chapters cover the historical aspects of anaesthesia in catalogue fashion and though the author admits the elementary character of the material in the third chapter, he places the heart between the lungs and the middle mediastinum.

In the second edition the author correctly referred to hydrogen ion concentration; in the current edition he has changed this to pH concentration. In eliciting a history from the patient he has nothing to state about getting evidence of cardiac disorders.

Not many physicians will agree with the author's contention that oxygen is the most important factor in the treatment of post-operative pneumonia, and no thoracic surgeon will countenance the recommendation of immediate bronchoscopy for patients with pulmonary collapse. In dealing with the signs of pulmonary collapse he omits to cite mediastinal displacement. He states that asthmatic patients take general anaesthesia badly whereas in fact they do well in between attacks. He fails to mention Ayre's tube for cleft palate operations, and in the chapter on "Intravenous Anaesthesia" he omits to warn of the dangers of intra-arterial injection. In the section on muscle relaxant drugs, he implies that patients with myasthenia gravis have an increased tolerance for drugs with quaternary ammonium groups.

Two new chapters, one on "Muscle Relaxants" and the other on "Controlled Hypotension" have been added, and the "Appendix of Useful Information" has been enlarged.

R. P. Harbord