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PHARMACOLOGICAL PROPERTIES OF 3-PHENYL-5 β DIETHYLAMINOETHYL-1,2,4-OXADIAZOLE

BY

B. SILVESTRINI AND C. POZZATTI

From the Department of Pharmacology, A. C. R. Angelini, F., Rome, Italy

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The general pharmacological properties of Oxolamine (3-phenyl-5β-diethylaminoethyl-1,2,4-oxadiazole) are described. The antitussive activity of this drug is more apparent in tests involving a diffuse stimulation of the bronchial tree than with electrical stimulation of the superior laryngeal nerve. These results suggest a predominantly peripheral mechanism of action. Oxolamine also possesses analgesic–anti-inflammatory, local anaesthetic and antispasmodic properties. The acute and chronic toxicities of Oxolamine are low, and the experimental results indicate the absence of side effects. The possibility that the antitussive activity is related to the other pharmacological properties is discussed.

Previous investigations (Silvestrini & Pozzatti, 1960) have shown that certain substituted aminoalkyl-1,2,4-oxadiazoles belonging to a homogeneous series (Palazzo, Strani, Tavella & Silvestrini, unpublished results) possess antitussive action. The most active compound among these derivatives, 3-phenyl-5β-diethylaminoethyl-1,2,4-oxadiazole (Oxolamine), was therefore submitted to a more detailed investigation.

METHODS

In the following tests mice were of the CF1 strain and weighed from 18 to 22 g; rats were of the Long-Evans strain and weighed from 150 to 200 g. Animals of both sexes were used. Unless otherwise stated, drugs were administered in aqueous solution as the hydrochloride.

Acute toxicity. The LD50 was calculated by the method of Weil (1952) on the basis of the death rate over 5-day periods following administration of the drug.

Chronic toxicity. One-month-old rats received daily 100 mg/kg Oxolamine hydrochloride injected intraperitoneally or 500 mg/kg Oxolamine citrate administered orally. The effect on the body weight was taken as the criterion of toxicity; the numbers of red and white corpuscles, haemoglobin concentration and behavioural changes were also noted. The animals were killed and various organs were taken for histological examination.

Acute behavioural effects. Mice received 25, 50, 75, 100 and 200 mg/kg Oxolamine hydrochloride by intraperitoneal injection. Effects on locomotor activity, muscle tone, righting reflex and on autonomic functions were assessed by the method of Irwin (1959).

Similar experiments were carried out on cats weighing from 2.0 to 2.5 kg.

Local irritation. Rats received a subcutaneous injection of Oxolamine (1 mg in 0.1 ml.) in the morning, afternoon and again next morning, and were sacrificed the following afternoon.

The same dose was injected into subcutaneous tissue of the external ear of rabbits which were observed for 3 days.

Antitussive activity. The acrolein inhalation test was used (Silvestrini & Maffii, 1959; Silvestrini & Pozzatti, 1960). The guinea-pigs were placed in pairs inside a transparent box, and 8 ml. of air saturated with acrolein vapour was blown into it. Each pair consisted of a control and of a treated animal. The number of coughs was counted over a 5-min period. The animals were removed and left for 3 min, after which they were observed for a further 5 min in which the number of coughs was again counted.

In a second set of experiments guinea-pigs were exposed for 5 min to a 2.8% (w/v) ammonia aerosol (Winter & Flataker, 1954). The guinea-pigs were selected according to the number of coughing fits observed during a single aerosol administration 6 hr before the test, and all animals with more than 16 fits or less than 4 were discarded. The drugs under examination were administered intraperitoneally 30 min before the tests were started. Weak coughing fits were given a value of 0.5 and strong fits of 1.

Cats were anaesthetized with pentobarbitone sodium (35 to 50 mg/kg intraperitoneally). The superior laryngeal nerve was stimulated electrically by the method of Domenjoz (1952). The drug was administered intravenously at the rate of 10 mg/kg in 2 min.

Analgesic–anti-inflammatory action. The method of Randall & Selitto (1957) was used in rats which had been starved overnight.

Analgesic activity. The hot-plate (Woolfe & Macdonald, 1944) and the phenylquinone (Siegmund, Cadmus & Lu, 1957) tests were applied to mice.

In the hot-plate test equal parts of acetone and ethylformate were brought to boiling point (55°) in a cylindrical container. This test was carried out only on animals which reacted to pain within 8 to 12 sec. All subsequent readings in the same animals were made 20, 40 and 60 min after the drugs had been administered. In the second test the compounds were injected 30 min before phenylquinone [0.25 ml. per animal of a 0.02% (w/v) phenylquinone solution in 5% (v/v) ethyl alcohol].

Local anaesthetic activity. This was tested in rabbits by finding the least concentration required to abolish the corneal reflex induced by touching the cornea (10 times consecutively) with a horse hair (Sollmann, 1918). Three drops of a buffered solution (*p*H 7) were instilled into the conjunctival sac, 3 min before the test.

Antispasmodic action in vitro. Strips of guinea-pig intestine were suspended in oxygenated Tyrode solution at 37° C. The dose of drug was found which caused a 50% inhibition of the contraction produced by acetylcholine chloride (0.02 μg/ml.), histamine hydrochloride (0.2 μg/ml.), barium chloride (50 μg/ml.) and dimethylphenylpiperazinium iodide (2 μg/ml.).

The tracheal chain preparation described by Castillo & De Beer (1947) was also employed. The contractions were induced with acetylcholine (0.5 μg/ml.).

Effect on the arterial blood pressure, respiration and on the response of the nictitating membrane. Adult cats were anaesthetized with chloralose (70 mg/kg) and pentobarbitone sodium (5 to 10 mg/kg), both given by intraperitoneal injection. The blood pressure was recorded with a mercury manometer connected to a cannula in the femoral artery. Respiration was recorded by a Marey tambour connected to the trachea. The effect of the drug was followed on the response of the blood pressure to doses of adrenaline (1 to 5 μg/kg intravenously), acetylcholine (0.5 μg/kg intravenously), dimethylphenylpiperazinium iodide (10 to 20 μg/kg intravenously) and to electrical stimulation of the distal end of the vagus cut in the neck. The response of the nictitating membrane was obtained by preganglionic stimulation of the cervical sympathetic nerve trunk (duration 4 min; frequency 10 to 30 sec; rectangular pulses of 1 msec duration).

The effect of the drug was also studied on the blood pressure of the unanaesthetized rat. A transistor microphone and air coupling were used whereby the pressure was progressively

increased until the blood pulsation at the tail ceased. The apparatus was designed and constructed in the workshop of the authors. It consists of a transistor amplifier of very low frequency response, and has filters to ensure non-sensitivity to all outside noise. A charcoal microphone was connected to the tail by means of a screw-graduated forceps.

Effect on intestinal peristalsis. The method was that of Stickney, Northup & Van Liere (1955). Mice were starved for 20 hr before the test. The drug was administered orally 30 min before the charcoal feed (5% w/v bone charcoal in 5% (w/v) aqueous solution of gum acacia). One hour later the mice were killed and the length of intestine traversed by the charcoal was measured.

Alarm reaction. Rabbits weighing from 2.3 to 2.5 kg were used. The hypothalamus was stimulated by the method of Monnier & Laue (1953), using a Grass stimulator model S4E (duration 3 sec; frequency 250/sec; pulse 1 msec).

Effect on conditioned response. The technique described by Cook & Weidley (1957) for the pole-climbing response in rats was employed. The buzzer was continued for 15 sec, or until the rat climbed the pole.

Anti-inflammatory activity. This was studied by the method of Randall, Selitto & Valdes (1957). Rats with about the same limb volume were specially selected for the test. Oedema was produced by the injection of brewer's yeast into the plantar surface of the rat's foot, and was estimated by measuring the change in volume of the limb (Buttle, D'Arcy, Howard & Kellett, 1957).

The granuloma test was also used and was carried out with cotton pellets weighing 5 mg (Meier, Schuler & Desaulles, 1950). Treatment was continued daily for one week, after which the animals were killed. Each granuloma was freed from the surrounding fatty tissue and weighed; it was weighed again after drying at 60° for 24 hr.

Antipyretic activity. The method used was that of Smith & Hamburger (1935). Sixteen hours before the test, each rat received an injection (10 ml./kg subcutaneously) of a 20% (w/v) brewer's yeast suspension. After the body temperature had remained constant for at least 1 hr, the animals were injected with the drug under test. The temperature was read every 30 min. The statistical significance of the values was calculated by the method of Wilcoxon (1949).

RESULTS

Acute toxicity

Table 1 shows the acute toxicity of Oxolamine and codeine in mice and rats.

By the intravenous route, Oxolamine and codeine were of similar toxicity; by other routes, Oxolamine was the less toxic. Oxolamine was lethal within 3 hr of administration, irrespective of the route.

TABLE 1

ACUTE TOXICITY IN MICE AND RATS

LD50 (mg/kg) with fiducial limits (P 0·05)

	Mouse				Rat	
Compounds	Intra-venously	Intra-peritoneally	Sub-cutaneously	Orally	Intra-peritoneally	Orally
Oxolamine hydrochloride	63 (55–72)	208 (179–241)	465 (371–583)	929 (772–1,120)	185 (138–248)	1,650 (1,370–2,000)
Oxolamine citrate	—	351 (318–387)	—	—	—	2,500
Codeine hydrochloride	67 (54–82)	125 (116–135)	286 (269–314)	365 (271–491)	—	—

Chronic toxicity

Neither Oxolamine hydrochloride (100 mg/kg) administered intraperitoneally each day for 45 days nor Oxolamine citrate (500 mg/kg orally) administered daily for 6 months affected the blood picture or body growth, or caused any pathological changes in the liver, kidney, spleen, heart or adrenals.

Local tolerance

The tests carried out on rats showed no local irritating effects. In rabbits there was a transient reddish zone at the site of injection. This local effect was less evident with Oxolamine citrate than with the hydrochloride.

Effects on behaviour

In mice, Oxolamine administered intraperitoneally in a dose of 50 mg/kg was without effect; 75 mg/kg reduced spontaneous locomotor activity and muscular strength; 100 mg/kg intensified these effects and produced ataxia; lethal doses caused convulsions.

In cats, Oxolamine, 50 mg/kg, had no apparent effect on the central nervous system or on the autonomic nervous system. The same dose of codeine provoked excitement of the sham rage type, disturbance of locomotion, mydriasis and vomiting.

The alarm reaction in the rabbit remained unaltered after 20 mg/kg Oxolamine injected intravenously. The same doses induced a transient alteration in the righting reflex. Morphine in a dose of 5 mg/kg intravenously increased the threshold stimulus.

The pole-climbing response in the rat remained unchanged after subcutaneous injection of 100 mg/kg of Oxolamine. A slightly delayed climbing time was observed, which suggests impaired muscular activity.

Antitussive action

The results are shown in Table 2.

An antitussive action was obtained with intraperitoneal injections of 1 mg/kg Oxolamine or with 2 mg/kg codeine. Oxolamine citrate produced the same inhibition of coughing as did the hydrochloride.

Oxolamine was twice as active as codeine in the test with ammonia aerosol. In this test the effective dose of both drugs was lower than that obtained with the same drugs in the acrolein test, possibly because coughing induced with ammonia aerosol was less intense. The results obtained in this series of experiments are shown in Table 3.

The action of Oxolamine on the cough reflex of the cat was as follows. After intravenous administration of 2 mg/kg a 50% inhibition was observed for 20 min in 1 of 4 animals; with 5 mg/kg the inhibition was 70 to 80% in 2 of 4 animals for 35 and 40 min respectively; and with 10 mg/kg the coughing response disappeared in 7 of 8 animals for more than 60 min.

TABLE 2

EFFECTS OF OXOLAMINE AND CODEINE ON ACROLEIN-INDUCED COUGHING IN GUINEA-PIGS

Control animals				Treated animals				
No. animals	No. coughs mean±s.e.	Compounds	mg/kg intraperitoneally	No. animals	No. coughs mean±s.e.	Inhibition %	t	p
5	15·4±2·6	Oxolamine	0·5	5	12·9±1·31	16	1·2	n.s.
15	13·0±1·27		1	15	9·26±0·88	29	3·43	0·01–0·001
16	13·93±1·52		2	16	8·53±0·72	39	3·1	0·01–0·001
10	13·8±1·29	Codeine	1	10	11·75±1·3	15	1·59	n.s.
10	14·4±1·56	hydrochloride	2	10	9·6±1·37	33	3·2	0·01–0·001

TABLE 3

EFFECTS OF OXOLAMINE AND CODEINE ON AMMONIA-INDUCED COUGHING IN GUINEA-PIGS

Number of coughs mean±s.e.

Compounds mg/kg intraperitoneally	No. animals	Before drug	After drug	Inhibition %	t	p
Saline	5	9·7±1·2	7·8±0·8	19·6	1·77	n.s.
Oxolamine hydrochloride 0·5	5	9·9±1·3	5·1±0·6	49·5	4·4	0·01–0·001
Codeine hydrochloride 1	7	8·86±2·2	4·92±0·8	44·5	3·0	0·02–0·01

TABLE 4

ANALGESIC-ANTI-INFLAMMATORY ACTIVITY IN RATS

(Randall and Selitto (1957) test)

Compounds	Doses mg/kg	No. animals	+30′ thresh. increase	t p	+90′ thresh. increase	t p	+150′ thresh. increase	t p
Oxolamine	10 subcutaneously	10	0	—	0	—	0	—
Oxolamine	20 subcutaneously	15	66	5·4 <0·001	30·7	3·37 0·01–0·001	20·7	3·58 0·01–0·001
Oxolamine	40 subcutaneously	15	58·7	2·89 0·01–0·001	53·7	3·42 0·01–0·001	29·4	1·59 n.s.
Oxolamine	40 orally	10	47	3·43 0·01–0·001	37	4·7 <0·001	28·5	4·2 <0·001
Aspirin	10 subcutaneously	5	0	n.s.	0	n.s.	0	n.s.
Aspirin	20 subcutaneously	20	42·3	4·75 <0·001	32	3·35 0·01–0·001	15·5	n.s.

Analgesic action

Results of the analgesic–anti-inflammatory test are shown in Table 4.

Oxolamine, both by subcutaneous injection and oral administration, displayed analgesic activity similar to that obtained with aspirin. However, Oxolamine in

doses up to 50 mg/kg subcutaneously was not analgesic in the hot-plate and phenylquinone tests, where morphine had been shown to be active in a dose of 2 mg/kg subcutaneously. Aspirin up to 80 mg/kg subcutaneously was without analgesic effects in the hot-plate test.

Local anaesthetic action

Oxolamine (1.0 to 1.5% w/v) produced anaesthesia of the rabbit cornea. Oxolamine citrate was as active in similar concentration.

Antispasmodic action

Oxolamine as the hydrochloride and citrate (5 μg/ml.) antagonized the effect of acetylcholine, histamine, dimethylphenylpiperazinium iodide, and barium chloride on the isolated intestine, and was about as active as papaverine in this test. In the tracheal chain preparation equiactive inhibitory doses of Oxolamine and papaverine were 2 μg/ml. and 1.2 μg/ml. respectively.

Arterial pressure and respiration

In the cat Oxolamine given intravenously in doses of 5 mg/kg produced a transient fall of the arterial pressure ranging from 5 to 10 mm Hg; 10 mg/kg caused the pressure to fall by 10 to 30 mm Hg and remain low for 3 to 8 min. There was a simultaneous increase in both the depth and frequency of respiration. Oxolamine up to 10 mg/kg did not modify the effects on the blood pressure of adrenaline, acetylcholine, dimethylphenylpiperazinium iodide, and of electrical stimulation of the vagus nerve, nor did it inhibit the contractions of the nictitating membrane.

In the conscious rat, Oxolamine given intraperitoneally up to 20 mg/kg did not modify the arterial pressure.

Action on intestinal motility

The results are given in Table 5.

Oxolamine had no effect in doses up to 50 mg/kg orally. Atropine (5 mg/kg) and codeine (25 mg/kg) delayed the progression of the charcoal meal.

TABLE 5
EFFECT ON PROGRESSION OF CHARCOAL MEAL IN MICE

Compounds mg/kg orally	No. animals	A Intestine length (cm) mean±s.e.	B Progression of charcoal (cm) mean±s.e.	Percentage of B/A	Comparison with controls t	Comparison with controls p	% Inhibition
Saline	20	28·6±0·6	18·8±1·27	65·7	—	—	—
Oxolamine 50	15	29·7±0·94	21·1±1·1	71	—	n.s.	0
Codeine 25	15	26·9±2	13±1·4	48·3	3·06	0·01–0·001	30·9
Codeine 50	5	28±1·56	11·9±2·6	42	2·45	0·05–0·02	37·3
Atropine 2·5	5	29·8±1·33	16·9±2·8	56·7	0·6	0·5–0·6	20·1
Atropine 5	5	27·1±1·3	6·4±0·8	23·6	4·7	<0·001	66

TABLE 6
ANTI-INFLAMMATORY ACTIVITY IN RATS

Compounds	Doses mg/kg subcutaneously	No. animals	Reduction of limb volume in ml. 2 hr	t p	4 hr	t p	6 hr	t p	24 hr	t p
Oxolamine	20	20	0·117	1·8 n.s.	0·127	1·75 n.s.	0·154	1·9 n.s.	0·049	n.s.
Oxolamine	40	20	0·157	2·12 <0·05	0·130	1·9 n.s.	0·110	n.s.	0·110	n.s.
Oxolamine	80	5	0·302	6·14 <0·001	0·198	7·2 <0·001	0·134	2·9 <0·002	0·080	n.s.
Phenyl-butazone	10	10	0·085	n.s.	0·098	n.s.	0·083	n.s.	0·035	n.s.
Phenyl-butazone	20	10	0·137	5·3 <0·001	0·132	2·49 <0·05	0·152	3·2 <0·01	0·167	n.s.
Phenyl-butazone	40	10	0·236	6·8 <0·001	0·274	3·8 <0·01	0·148	4·13 <0·01	0	—
Aspirin	60	20	0·030	n.s.	0	—	0	—	0	—
Aspirin	120	29	0·084	n.s.	0·178	2·28 0·05–0·02	1·188	3·6 0·01–0·001	0·067	n.s.

Anti-inflammatory and antipyretic activity

The results are shown in Table 6. The anti-inflammatory activity of Oxolamine was one-half to one-third of that possessed by phenylbutazone, but more active than that of aspirin, which, according to our experiments, required doses above 100 mg/kg subcutaneously.

Oxolamine in doses of 40 mg/kg as daily subcutaneous injections was inactive in the granuloma test in normal rats.

Oxolamine had an antipyretic action in oral doses of 200 mg/kg in rats; phenylbutazone was active at the same dose and aspirin showed an activity with 100 mg/kg orally.

DISCUSSION

The antitussive action of Oxolamine was studied in tests which differed in the type of stimulus applied to produce cough. In tests on guinea-pigs, cough was obtained by diffuse stimulation of the respiratory tract with irritant aerosols: Oxolamine was effective in small doses. In tests on anaesthetized cats, cough was obtained by electrical stimulation of the superior laryngeal nerve: Oxolamine was not effective except in large doses. The difference in sensitivity of the cough reflex to Oxolamine in the two tests suggests that the drug acts mainly on receptors in the lung and, to a lesser degree, on the cough centre. Oxolamine did not depress respiration, and in the tests on guinea-pigs it was more active than codeine.

The analgesic–anti-inflammatory action of Oxolamine, noted in the Randall & Selitto (1957) test, may be compared with that of aspirin. For example, Oxolamine showed undoubted anti-inflammatory and antipyretic activity. Oxolamine was inactive in the hot-plate test, which, according to Winder (1959), is more likely to demonstrate the analgesic activity of centrally acting drugs. Furthermore, the absence of excitation in the cat typical of morphine and codeine; the absence of

effects on the alarm reaction in the rabbit caused by central depressants and tranquillizers (Napolitano & Longo, 1957; Silvestrini, 1958; Silvestrini & Kohn, 1958), and the absence of effects on the conditioned response of the rat, such as may be seen with central analgesics (Blumberg & Dayton, 1959), strongly suggest that the analgesic action of Oxolamine is not central.

The antispasmodic action as studied on the isolated intestine of guinea-pig was unspecific, since Oxolamine inhibited contractions induced by acetylcholine, histamine, barium chloride, and dimethylphenylpiperazinium iodide. In cats, Oxolamine appeared to be devoid of antihistamine, anticholinergic, antiadrenergic, and ganglionic-blocking activity.

Oxolamine is a drug of low toxicity and would be expected to have few side-effects. Rats withstood the daily administration of a dose equivalent to one-half LD50 with no evident effect. In acute experiments, large doses of Oxolamine appeared to be without effect on behaviour, blood pressure, respiration, and intestinal motility.

The results of the present tests suggest that Oxolamine might be more effective in suppressing cough associated with inflammatory changes in the bronchial tree than cough arising exclusively from stimulation of sensory nerves in the larynx. On this view, the antitussive action would depend mainly on the anti-inflammatory and spasmolytic effect of the drug on structures in the lung, and to a lesser extent on depression of the cough centre. Further evidence, both clinical and pharmacological, is being sought to substantiate this hypothesis.

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