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The pharmacology of ambenoxan (2-(3′,6′-dioxaheptyl)-aminomethyl-1 : 4-benzodioxane), a centrally acting muscle relaxant

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Summary

1. The intravenous, subcutaneous and oral toxicity of ambenoxan in mice is reported.

2. Ambenoxan is a centrally acting skeletal muscle relaxant shown to be effective in mice, rats, rabbits, dogs and monkeys without loss of the righting reflex.

3. It had no peripheral neuromuscular blocking properties.

4. Decerebrate rigidity was depressed or abolished in the rabbit.

5. The effects of strychnine, leptazol, or tremorine were not antagonized.

6. In common with other depressants of the central nervous system, ambenoxan prolonged the sleeping time of hexobarbitone.

7. Ambenoxan had no local anaesthetic properties.

8. In the anaesthetized cat the drug lowered the blood pressure and reduced the pressor response to adrenaline but not to noradrenaline.

Introduction

Bovet & Simon (1937) described the sympatholytic and sedative properties of a series of aminomethyl benzodioxanes. In 1959 Klupp & Streller reported on the muscle relaxant effect of 2-(3′-methoxypropylaminomethyl)-1 : 4-benzodioxane (Quiloflex). Rathburn, Henderson, Kottau & Keller (1958) and Slater & Jones (1958) have shown that the 1 : 4-benzodioxane derivatives butamoxane, ethoxybutamoxane and chloroethoxybutamoxane are potent central nervous system depressants with a tranquillizing effect. The adrenolytic and depressant effect on the central nervous system of a series of 2-substituted aminomethyl-1 : 4-benzodioxanes has recently been described by Green, Shapero & Wilson (1969). One of the series, ambenoxan (2-(3′,6′-dioxaheptyl) aminomethyl-1 : 4-benzodioxane) was selected for more detailed pharmacological investigation, the results of which are now reported.

Methods

Acute toxicity

Groups of albino mice of both sexes were given ambenoxan at various dose levels by the oral, subcutaneous and intravenous routes. The animals were observed

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daily for 7 days, and the LD50 values calculated by the method of Litchfield & Wilcoxon (1949).

A further series of toxicity experiments was carried out in which the compound was infused intravenously into male albino mice (0·3 ml/min) and rabbits (4 ml/min) and the dose causing the first convulsive twitch and respiratory arrest (no respiratory movement for 15 s) was recorded.

Muscle relaxant properties

Ambenoxan was given orally, intraperitoneally, subcutaneously and intravenously to conscious rats, rabbits, dogs and rhesus monkeys, and the muscle relaxant effect assessed by observing head drop and inability of the limbs to support the body.

Action on the neuromuscular junction

Rabbits were anaesthetized with urethane (1 g/kg intravenously) and the sciatic nerve of one leg exposed and cut between ligatures. The peripheral end of the sciatic nerve was stimulated with supramaximal shocks (1 ms duration, square wave) every 10 s at 2–4 V and recordings made of the contractions of the gastrocnemius muscle. Injections were given through a cannula in the jugular vein.

Effect on decerebrate rigidity in the rabbit

Rabbits were deeply anaesthetized with ether and decerebration carried out by sectioning the mid-brain and removing the cerebral hemispheres by suction. One hour elapsed before the drug was administered through a marginal ear vein. This was found to be sufficient time for the animal to eliminate the ether and recover from shock. Animals were only used if they showed pillar-like rigidity of the limbs, arched back, head retraction and normal respiration.

Anticonvulsant activity

Groups of five male albino mice (20–25 g) were injected subcutaneously with ambenoxan 20 mg/kg. Control animals received a similar volume of saline. After 30 min, treated and control groups were slowly infused through a tail vein with either 0·5% leptazol or 0·1% strychnine hydrochloride at a rate of 0·03 ml/min. The anticonvulsant effect was determined by the method of Orloff, Williams & Pfeiffer (1949).

Action against tremorine

The effect of ambenoxan against tremorine (1-4-dipyrrolidino-2-butyne) was investigated by the method described by Everett (1956). Groups of five male albino mice were dosed orally with ambenoxan, benzhexol hydrochloride, or saline as control; 15 min later they were injected intraperitoneally with 15 mg/kg of tremorine. The animals were observed at 15 min intervals for a period of 60 min.

Potentiation of hexobarbitone

Ten male mice (20–25 g) were injected intraperitoneally with 100 mg/kg of sodium hexobarbitone alone. A similarly treated group of animals received ambenoxan simultaneously by the subcutaneous route. As the mice lost their righting

reflex they were placed on their backs on trays in a Perspex cabinet which served to maintain a constant temperature. The duration of sleep was assessed as the time required for the mice to regain their righting reflex.

Effect on blood pressure

Conscious rabbits. Rabbits were trained to sit quietly in a warm draught-free room while the systolic pressure in the ear artery was determined by the capsule method of Grant & Rothschild (1934). After the control blood pressure had been established three rabbits were injected intravenously with 5 mg/kg, and a further three subcutaneously with 25 mg/kg of ambenoxan. The arterial pressure was re-determined 5, 30, 60 and 90 min after injection.

Anaesthetized cats. Animals of either sex weighing 2–4 kg were anaesthetized intraperitoneally with 48 mg/kg of pentobarbitone sodium. Blood pressure was recorded from a carotid artery using a mercury manometer. Drugs were injected through a cannula in the jugular vein.

The effects on standard adrenaline and noradrenaline pressor responses were investigated immediately, and 30 min after, intravenous doses of ambenoxan.

The effect on sympathetic ganglia was determined by recording contractions of the nictitating membrane following stimulation of the preganglionic fibres with maximal square wave shocks at a frequency of 10 Hz, 1 ms duration for 15 s at 2 V.

Local anaesthetic properties

Surface anaesthesia was determined in albino rabbits. 0·5 ml of a 2% solution was instilled into the conjunctival sac and the eyelid closed for 30 s. Loss of the blink reflex was evaluated by applying pressure with a fine camel hair brush to the cornea at regular intervals. The left eye was treated with saline and served as a control.

Conduction anaesthesia was investigated in male albino mice by the tail clip method of Bianchi (1956). 0·1 ml of a 2·5% solution was injected subcutaneously about 1 cm from the root of the tail ; 15 min later and thereafter at 15 min intervals they were tested for loss of response to pain.

Spasmolytic properties

Segments of guinea-pig ileum were suspended in 7 ml of aerated Tyrode solution at 32° C. Standard submaximal responses to acetylcholine or histamine were established before the addition of the test compound to the bathing fluid. Following a 30 s contact time, either acetylcholine or histamine was added to the bath and left for a further 30 s before washing. Spasmolytic activity was expressed as % inhibition to the standard responses.

Drugs

Doses of the drugs used are expressed as the salts. Adrenaline bitartrate, (−)-noradrenaline bitartrate, sodium hexobarbitone, tremorine hydrochloride, leptazol hydrochloride, benzhexol hydrochloride, acetylcholine chloride and histamine acid phosphate.

Results

Acute toxicity

The LD50 values for ambenoxan in the mouse are given in Table 1. The drug is approximately twice as toxic orally as it is subcutaneously, which suggests that the compound is effectively absorbed by the oral route. Drug effects were apparent five minutes after administration by the oral or subcutaneous route. In the early stages the mice became lethargic but could be roused, later they failed to respond to external stimuli. Respiration was at first rapid and deep, then became slow and shallow and, with lethal doses, death ensued within a few hours. Intravenously, ambenoxan sometimes caused a brief opisthotonos. Death was due to respiratory failure.

Table 2 shows that following slow intravenous infusion in mice, the toxicity of ambenoxan was reduced 3·5 times compared with a single rapid dose (Table 1). Deaths, when they occurred, were due to respiratory depression whether the drug was injected slowly or rapidly. The dose causing opisthotonos (convulsive dose) was slightly more than one half of the dose causing respiratory arrest.

The convulsive dose in rabbits was approximately one fourth of the dose causing respiratory arrest (Table 2). All the rabbits survived the maximum dose infused.

Muscle relaxant effect

Rat. Ambenoxan, 50–100 mg/kg orally or subcutaneously, caused splaying of the limbs with low body posture. This effect was not preceded by ataxia or excitation. Onset of action was within 15 min and lasted 2–4 hr. With 100 mg/kg respiration was shallow and rapid, and the ears were pink, suggesting a vasodilator effect.

Rabbit. Similar effects to those observed in the rat were obtained with intravenous (2·5 and 5 mg/kg) and subcutaneous (25 and 50 mg/kg) doses of ambenoxan. The higher dose of 10 mg/kg intravenously provoked brief extensor spasm followed by muscle relaxation. With 50 mg/kg subcutaneously two out of eight rabbits developed convulsions which could be initiated by noise or prodding.

TABLE 1. *Acute toxicity of ambenoxan in mice*; 95% *confidence limits in parentheses*

Sex	Route	LD50 (mg/kg)
Male	Intravenous	24·5 (21·9–27·4)
Female	Intravenous	20·2 (16·2–25·2)
Male	Subcutaneous	920 (829–1,021)
Female	Subcutaneous	830 (715–963)
Male	Oral	410 (330–508)
Female	Oral	300 (242–372)

TABLE 2. *Toxic effect of an intravenous infusion of ambenoxan into mice and rabbits*

Species	Convulsive dose (mg/kg)	No. of animals convulsing/No. tested	Respiratory arrest (mg/kg)	Mortality
Mice	50·5±0·77	6/8	84·06±3·47	4/8
Rabbits	12·8±1·25	5/5	43·6 ±0·70	0/5

The infusion rate for mice of a 0·25% solution was 0·3 ml/min and for rabbits of an 0·5% solution 4 ml/min.
Values are means and standard errors.

Table 3 shows that the duration of muscle relaxation in the rabbit following an intravenous or subcutaneous injection of ambenoxan increased in relation to dose.

The intravenous injection of 25 mg/kg of mephenesin was immediately followed by ataxia and muscle relaxation which lasted approximately 35 min in the three rabbits tested, and at 50 mg/kg intravenously two of the three rabbits tolerated the side position for 2–5 min. The duration for both doses was approximately 35 min.

Dog. Ambenoxan given intravenously (2·5 to 5 mg/kg), subcutaneously (6·25 to 25 mg/kg) and orally (12·5 to 75 mg/kg) induced muscle relaxation without loss of the righting reflex. The intensity and duration of the effect was dose and route dependent; recovery after intravenous injection took 30–60 min, and after subcutaneous or oral administration 1–4 hr.

Monkey

Injection of 5 mg/kg intraperitoneally in two rhesus monkeys produced effects within 5–10 min and lasted 50–70 min. The principal effect was loss of muscle tone, changes in posture and facial appearance, such as the drooping of the lower jaw.

Action on the neuromuscular junction

In the anaesthetized rabbit 5 mg/kg of ambenoxan given intravenously had no effect on neuromuscular transmission, whereas (+)-tubocurarine in doses of 0·4 mg/kg (i.v.) blocked the responses of the gastrocnemius muscle to stimulation of the sciatic nerve.

Effect on decerebrate rigidity

An intravenous injection of 5 mg/kg of the drug was effective in abolishing decerebrate rigidity and tremor in the rabbit (Table 4).

Anticonvulsant activity

Ambenoxan 20 mg/kg subcutaneously failed to raise the leptazol and strychnine convulsant threshold.

TABLE 3. *Duration of muscle relaxation in rabbits*

Dose of Ambenoxan (mg/kg)	Route	Duration (min)
2·5	Intravenous	23±2 (8)
5	Intravenous	39±8 (8)
10	Intravenous	54±3 (7)
12·5	Subcutaneous	71±3 (7)
25	Subcutaneous	99±3 (14)
50	Subcutaneous	203±7 (8)

The numbers in parentheses represent the number of animals used. Values are means and standard errors.

TABLE 4. *Effect of intravenous ambenoxan on decerebrate rigidity in rabbits*

No. of animals	Dose (mg/kg)	No. showing loss of rigidity and tremor	Duration (min)
3	2·5	1	30
3*	5	2	20–90

* One died immediately after the injection.

Effect against tremorine

Benzhexol 5 mg/kg subcutaneously was effective in suppressing continuous tremor and the parasympathomimetic effect of tremorine, whereas ambenoxan 50 mg/kg had no effect.

Potentiation of hexobarbitone

The mean value (with standard error) for sleeping time in mice following the injection of hexobarbitone 100 mg/kg alone was $26 \pm 4{\cdot}3$ min, and for hexobarbitone 100 mg/kg plus ambenoxan 5 mg/kg it was $42{\cdot}6 \pm 6{\cdot}0$ min. The difference in sleeping time between the two groups was statistically significant ($P<0{\cdot}05$). A subcutaneous dose of 5 mg/kg of ambenoxan produced no overt depressant effect in mice.

Effect on the blood pressure of the conscious rabbit

In two out of three rabbits 5 mg/kg of ambenoxan intravenously caused a rapid transient fall in blood pressure which returned to its original level within 5 min. 25 mg/kg given subcutaneously to another three rabbits had no effect.

Effect on the blood pressure of the anaesthetized cat

Intravenously ambenoxan (0·5 and 1 mg/kg) caused an immediate fall of 25 and 33 mm Hg respectively (mean of three cats at each dose level). The blood pressure returned to normal within 60 to 150 min.

Effect on the pressor responses to adrenaline and noradrenaline

Immediately following an intravenous injection of 0·5 and 1 mg/kg of ambenoxan the pressor response to adrenaline was reduced by approximately 60%, whereas the effects of noradrenaline were unchanged. After 30 min the pressor response to adrenaline was normal.

Effect on sympathetic ganglia

Contractions of the cat nictitating membrane were not inhibited by the intravenous administration of 0·5 or 1 mg/kg ambenoxan.

Local anaesthetic activity

Five minutes after instillation of 0·5 ml (20% solution) of ambenoxan into conjunctival sacs, one out of three rabbits failed to respond to stimulation of the cornea. Local anaesthesia lasted only 15 min.

Conduction anaesthesia could not be demonstrated in mice following subcutaneous injection into the tail of 0·1 ml (2·5% solution) of the compound.

Spasmolytic properties

Ambenoxan (50 μg) reduced the effect of acetylcholine (0·22–2 μg) and histamine (1·2–10 μg) on the isolated guinea-pig ileum by 50%. A similar effect occurred after papaverine (50 μg).

Discussion

Ambenoxan has been shown to produce skeletal muscle flaccidity without loss of the righting reflex when administered orally or parenterally to rats, rabbits, dogs and monkeys.

In the rabbit the muscle relaxant effect following ambenoxan was similar to that produced by mephenesin. However, the splayed limbs and loss of postural control contrasted with the lethargic, ataxic gait seen after mephenesin, suggesting that the mode of action of these two compounds is different. Furthermore, unlike mephenesin (Berger & Bradley, 1946) and meprobamate (Berger & Ludwig, 1950) ambenoxan did not antagonize convulsions induced by leptazol or strychnine. In this respect ambenoxan behaved like orphenadrine and phenyramidol (Cronheim, 1961). In common with other centrally acting muscle relaxants ambenoxan caused paralysis of the ascending type and prolonged time of sleep produced by hexobarbitone.

The hypotonia induced by ambenoxan in the rabbit was sometimes preceded by a brief extensor spasm following a rapid intravenous injection. This effect did not originate from anoxia, for, as shown in Table 2, mice infused intravenously with 0·25% solution of ambenoxan exhibited the convulsant effect before respiratory arrest. However, the effect was not pronounced and only occurred with high doses in rabbits and mice. It was not observed in rats, dogs or monkeys with the doses used. This effect may be linked with an observation of Meldrum & Bhargava (1968), who, in a study of the convulsant and muscle relaxant properties of ambenoxan, reported that with high intravenous doses, transient motor and electroencephalographic epileptic activity preceded muscle hypotenia. Berger (1947) showed that rapid intravenous injection of mephenesin in the rabbit produced rigidity followed by muscle relaxation.

Ambenoxan did not produce obvious sedative activity in the rhesus monkey, rabbit or the rat. Failure of the dog to react to sound suggests that a sedative phase may be present. Differences in metabolism may account for the species variation.

The possibility of a curare-like effect in the rabbit was eliminated when it was found that ambenoxan (5 mg/kg) did not effect contraction of the gastrocnemius muscle following stimulation of the sciatic nerve. Further, experiments in mice by Cymbalist & Shapero (unpublished) showed that the muscle paralysis induced by ambenoxan was not reversed by neostigmine, thus confirming the lack of a curare-like action on skeletal muscle.

The muscle relaxant properties of ambenoxan cannot be attributed to its hypotensive effect. In the conscious rabbit 5 mg/kg intravenously caused a prolonged depression of skeletal muscle tone with a negligible effect on blood pressure.

These findings suggest that ambenoxan exerts its effects via an action on brain and/or spinal mechanisms. This conclusion is supported by the fact that ambenoxan abolished the extensor tonus following mid-brain decerebration in the rabbit.

Preliminary human pharmacological studies have been carried out and results confirm that the drug exerts a relaxant effect on skeletal musculature with intravenous and oral doses insufficient to cause undesirable effects.

Ambenoxan may therefore be of value in the treatment of spastic disorders resulting from upper motor neurone damage in the brain or spinal cord, disseminated sclerosis, spastic diplegia and spastic hemiplegia.

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