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Myoclonic seizures in the mouse induced by alphaxalone and related steroid anaesthetics

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Abstract—The anaesthetic steroids alphaxalone. 5β-alphaxalone and pregnanolone each caused myoclonic jerks in mice in a dose-related manner between 4 and 16 mg kg^{-1} i.v. There was no loss of righting reflex at these doses. The veterinary product Saffan, which contains alphaxalone and alphadalone, also caused myoclonic jerks at 2 mg kg^{-1} i.v., and a loss of righting reflex at doses of 4 mg kg^{-1} and above. These effects appear to be unrelated to the wide spectrum of potencies at the $GABA_A$ receptor complex of the three individual steroids as potentiators of muscimol, or as attenuators of picrotoxin.

The steroidal anaesthetic, alphaxalone, potentiates responses to the $GABA_A$ receptor agonist, muscimol, in the rat cuneate nucleus (Harrison & Simmonds 1984) and prolongs the open times of Cl^- channels operated by the $GABA_A$ receptor (Barker et al 1987). These actions are similar to those of barbiturates. However, the barbiturates differ as to whether they possess anticonvulsant as well as anaesthetic actions, e.g. pentobarbitone is not anticonvulsant at sub-anaesthetic doses, whereas phenobarbitone is anticonvulsant at doses that are not excessively sedative. These two barbiturates have different profiles of interaction with the $GABA_A$ receptor complex (Harrison & Simmonds 1983). Their relative potencies as potentiators of muscimol correlate well with their relative anaesthetic potencies. But, at equi-effective concentrations for a small potentiation of muscimol, phenobarbitone reduced the antagonism of muscimol by picrotoxin, whereas pentobarbitone did not (see Simmonds 1986). It was therefore suggested that this latter phenomenon might be relevant to the anticonvulsant action of phenobarbitone.

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Similar studies of the effects of a series of steroids related to alphaxalone on the rat cuneate nucleus have shown an analogous distinction between the structure/activity profile for potentiation of muscimol, and that for the reduction in the potency of picrotoxin as a muscimol antagonist (Turner 1987). Therefore we selected for study as potential anticonvulsants three steroids that were distinctly different from each other in terms of their interactions with the $GABA_A$ receptor complex. They were alphaxalone (3α-hydroxy-5α-pregnane-11,20-dione), pregnanolone (3α-hydroxy-5β-pregnane-20-one) and 5β-alphaxalone (11-keto-pregnanolone). Their potencies as potentiators of muscimol were alphaxalone > pregnanolone > 5β-alphaxalone (Simmonds & Turner 1987). At equi-effective concentrations for a small potentiation of muscimol, alphaxalone had no effect on picrotoxin potency, whereas pregnanolone caused a large reduction that was substantially greater than that previously reported for phenobarbitone (Turner 1987). 5β-Alphaxalone also caused a large reduction in picrotoxin potency at concentrations that produced little or no potentiation of muscimol.

We therefore started with the aim of comparing in-vivo the sedative and anticonvulsant actions of these steroids. A decrease in spontaneous locomotor activity is a sensitive measure of sedative drug effects and this was measured in a holeboard (File & Wardill 1975). Pilot experiments were conducted to determine the time at which the effects of alphaxalone (1 mg kg^{-1} i.v.) were maximal in the holeboard.

The results showed a peak effect 20 min after i.v injection, but rather than finding a sedative effect there was an *increase* in motor activity (mean number of beam breaks, controls 213·6, alphaxalone 315·0). In addition, pilot experiments with higher doses revealed the incidence of myoclonic jerks during a 20 min observation period.

The experiments were therefore redesigned to study the incidence of myoclonus following i.v. administration of the three steroids. Where the incidence was low, the mice were then placed in a holeboard to measure their locomotor activity. Since the direction of the steroid effects on seizures was unexpected the importance of the route of administration was investigated by studying the effects of alphaxalone after i.p. administration.

Finally, an additional group of mice was studied after i.v. administration of Saffan, the commercial formulation of alphaxalone + alphadalone. The addition of alphadalone is to improve solubility of alphaxalone, but we wished to determine whether it had any protective effect against seizures.

Methods

Animals. Male mice (Bantin & Kingman outbred strain) approximately 35 g were housed in groups with food and water freely available.

Drugs. Alphaxalone (kindly donated by Glaxo Group Research), pregnanolone (Sigma) and 11-keto-pregnanolone (Sigma) were dissolved in polyethylene glycol 400 (Sigma). Saffan (Glaxovet) was the veterinary product.

Apparatus. The holeboard was a wooden box with 40 cm square floor and walls 27 cm high. The interruption of infrared photobeams in the walls of the box (1·5 cm from the floor) provided an automated measure of locomotor activity.

Procedure

Experiment 1. Fifty-six mice were randomly allocated, eight to each of the following groups: vehicle control; 0·5, 1, 2, 4, 8 and 16 mg kg^{-1} alphaxalone. Each mouse was observed for 20 min following i.v. injection and the incidence and duration of myoclonic jerks was recorded. At the end of this observation each mouse was placed in the holeboard for a 7·5 min trial. Mice that were experiencing a prolonged episode of myoclonus were not tested in the holeboard. All testing took place between 1400 and 1600h.

Experiment 2. Forty-eight mice were randomly allocated, six to each of the following groups: vehicle control; 5-β-alphaxalone (4, 8 and 16 mg kg^{-1}); vehicle control; pregnanolone (4, 8 and 16 mg kg^{-1}). Each mouse was observed for 20 min following i.v. injection and the incidence and duration of myoclonus was recorded. In this experiment the seizures were too severe to permit testing in the holeboard.

A further 24 mice were randomly allocated, six to each of the following groups; vehicle control; alphaxalone (4, 8 and 16 mg kg^{-1}). All injections were given intraperitoneally and the mice were observed for the following 20 min for the incidence and duration of myoclonus.

All testing took place between 1400 and 1600h.

Experiment 3. Thirty-two mice were randomly allocated, six or seven to each of the following groups: vehicle control; Saffan (1, 2, 4, 8 and 16 mg kg^{-1}). All injections were intravenous and the mice were observed for the next 20 min for the incidence of myoclonus. At the end of this period each mouse was given a 7·5 min trial in the holeboard. Once again mice experiencing prolonged myoclonus were excluded from the holeboard test. All testing took place between 1400 and 1600h.

Results

Table 1 shows the incidence and duration of myoclonic jerks following i.v. injection of the three steroids. There was a significant incidence of myoclonus from 4 mg kg^{-1} alphaxalone and there was a significant dose-related increase in the duration of myoclonus. The intraperitoneal route gave almost identical results to the intravenous route (see Table 1). Both pregnanolone and 5-β-alphaxalone produced seizures in a significant number of mice, from 4 and 8 mg kg^{-1}, respectively. The duration of the seizures produced by 16 mg kg^{-1} pregnanolone were twice those produced by the same dose of the other steroids.

As can be seen from Table 2 i.v. Saffan also produced a significant incidence of myoclonic jerks. In addition, from

Table 1. Incidence of myoclonic jerks and mean ± s.e.m. duration of the activity.

Dose (mg kg^{-1})	Number of mice with myoclonus	Duration of myoclonus (s)
Alphaxalone (i.v.)		
0	0/8	
0·5	0/8	
1	0/8	
2	4/8*	
4	4/8*	525 ± 110
8	6/8**	656 ± 125
16	6/8**	1282 ± 153
Alphaxalone (i.p.)		
0	0/6	
4	6/6**	756 ± 145
8	5/6**	884 ± 86
16	5/6**	1268 ± 118
5-β-Alphaxalone (i.v.)		
0	0/6	
4	3/6	798 ± 178
8	6/6**	1140 ± 188
16	5/6**	1338 ± 87
Pregnanolone (i.v.)		
0	0/6	
4	4/6*	778 ± 68
8	5/6**	1520 ± 92
16	5/6**	2885 ± 148

Groups significantly different from controls. *$P < 0·05$, **$P < 0·01$. Fisher exact probability test.

Table 2. Incidence of myoclonic jerks and immobility following i.v. administration of Saffan.

Dose (mg kg^{-1})	Myoclonus	Immobility
0	0/7	0/7
0·5	1/7	0/7
1	3/6	0/6
2	5/7**	2/7
4	5/6**	3/6
8	4/6*	3/6

Significantly different from controls. *$P < 0·02$, **$P < 0·01$. Fisher exact probability test.

4 mg kg^{-1} upwards several mice lost the righting reflex and lay immobile.

Table 3 shows the mean motor activity scores for the mice tested in the holeboard after i.v. alphaxalone (0·5–16 mg kg^{-1}) or Saffan (1–4 mg kg^{-1}). Saffan caused immobility and loss of righting (indicating an anaesthetic action) at doses of 8 and 16 mg kg^{-1} and so it was not possible to test the mice in the holeboard. As can be seen from Table 3, alphaxalone tended to increase motor activity, but this was not significant. The trend with Saffan was for a decrease in motor activity, but at doses up to 4 mg kg^{-1} it was not significant.

Table 3. Mean (±s.e.m.) motor activity scores for mice tested 20 min after i.v. administration of alphaxalone or Saffan.

Alphaxalone (mg kg^{-1})		Saffan (mg kg^{-1})	
0	213·6 ± 21·7	0	235·4 ± 14·4
0·5	250·5 ± 33·1		
1	291·6 ± 35·9	1	218·7 ± 20·9
2	243·9 ± 26·1	2	187·8 ± 31·5
4	251·3 ± 14·2	4	195·4 ± 23·8
8	209·9 ± 15·9		
16	273·8 ± 27·6		

F (6,50)= 1·31; F (3,20) = 0·95.

Discussion

All three of the steroids, as well as Saffan, caused myoclonic jerks in doses that were insufficient to cause a loss of righting reflex. This effect of alphaxalone was seen following either intravenous or intraperitoneal injection. There was little difference between the threshold doses of these steroids for the myoclonic activity, although, at the higher doses tested, the duration was greater for pregnanolone than for alphaxalone or 5β-alphaxalone. The lack of a sedative effect in mice of the individual steroids at doses up to 16 mg kg^{-1} i.v. accords with the much higher doses that were needed to induce a 25 min loss of righting reflex. Specifically, alphaxalone (64 mg kg^{-1}), 5β-alphaxalone (59 mg kg^{-1}) and pregnanolone (27 mg kg^{-1}) (Atkinson et al 1965). The much lower dose of Saffan required to produce loss of righting in our study is likely to be due to a superior absorption of alphaxalone from the Saffan formulation. It is unlikely that the vehicle we used would have reduced the sedative effects of the steroids, since the effects of such solvents are, if anything, to potentiate muscimol (Simmonds 1981). However, the formulation of these steroids clearly has a significant effect on their anaesthetic efficacy and a pregnanolone emulsion has been reported to cause loss of righting in mice with an ED50 of 5·25 mg kg^{-1} (Hogskilde et al 1987). Thus the rate of onset of anaesthesia is likely to be significantly affected by the formulation, and this is also likely to determine whether or not myoclonic seizures are detected.

The clear myoclonic activity that we observed was unexpected. The data sheet for Saffan (Glaxovet) makes no mention of such effects in cats or monkeys, for which the product is recommended as an anaesthetic. However, the Extra Pharmacopeia (Martindale) reports involuntary muscle movements with alphaxalone in man, and the Data Sheet Compendium for Althesin (Glaxo, alphaxalone + alphadalone) lists muscle twitching and generalized convulsions as side effects.

As a consequence of these findings, our original hypothesis that the ability of a compound to reduce the potency of picrotoxin as an antagonist at the $GABA_A$ receptor complex would correlate with anticonvulsant properties has not been supported for these steroids. There is no evidence that the relative potencies of the three steroids either as potentiators of muscimol, or as attenuators of picrotoxin correspond in any way with their potencies at inducing seizures. Any anticonvulsant potential that these steroids may possess was clearly inadequate to overcome their own convulsant properties. However, it is possible that the seizure activity is related to actions at sites outside the GABA receptor complex.

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