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The pharmacology of *Avena sativa*

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The pharmacology of *Avena sativa* has been investigated in laboratory animals following a report that tincture of *Avena sativa* reduced the craving for cigarettes in man. The tincture, evaporated to dryness, re-constituted in an equal volume of water and administered by stomach tube or intraperitoneal injection, antagonized the antinociceptive effect of morphine in two separate tests (hot-plate and tail flick). Compared with animals made dependent on morphine alone, mice pretreated with repeated injections of morphine plus extract passed a smaller number of stools and tended to jump less after administration of nalorphine. The pressor response to intravenously administered nicotine in urethane-anaesthetized rats was also antagonized by prior administration of *Avena sativa*. However, the aqueous extract prepared from the tincture did not affect the seizure threshold to bemegride or nicotine or the sleeping time induced by barbitone sodium.

Following a report that a decoction of the common oat (*Avena sativa*), used successfully in India to "cure the opium habit", also reduced the craving for tobacco, Anand (1971) studied the effect of oral administration of an alcoholic tincture of oats on the number of cigarettes smoked in a group of bronchial patients. The drug seemed to reduce the craving for, and the number of, cigarettes smoked per day. As an adjunct to clinical trials of similar preparations of *Avena sativa* in the Dundee Anti-Smoking Clinic, we have attempted to assess the biological activity of such extracts in laboratory animals. Since the tincture had previously been recommended as a cure for the opium habit (Clarke, 1925), the first experiments were designed to investigate whether it affected the analgesic potency of morphine or modified the development of physical dependence on morphine in simple pharmacological tests. Our preliminary findings indicated that the tincture antagonized the effect of morphine. This paper presents our results with different preparations of the tincture in several pharmacological tests.

METHODS

Preparation of tincture of Avena sativa

Fresh green plants of the common oat were selected at the stage when the flowering stem was just emerging. The roots were discarded and the green stalks were chopped into short pieces. Three parts of chopped plant were macerated with 10 parts of 90% w/v ethanol for three days in 5 litre flasks, with occasional agitation. The resultant alcoholic tincture was separated by decantation and filtration through Whatman No. 1 paper. Residual tincture was expressed from the plant debris by squeezing in muslin and was subsequently filtered. The tincture was stored in amber glass bottles at room temperature. Because of the encouraging results obtained in preliminary experiments, steps were taken to acquire tinctures prepared from oat seed, oat plants at various stages of development and from plants grown in

different geographical locations. Details of the various tinctures prepared are given in Table 3 which also presents the ED50's of the various extracts determined against morphine on the hot-plate. The ED50's were determined graphically after administration of graded doses of each extract to groups of 4 mice.

Administration to rats or mice

Aliquots of 25 ml of the tincture were evaporated to dryness (<40°) and stored at −20° until immediately before testing. The residue was re-constituted in 25 ml distilled water and filtered once through Whatman No. 1 paper. Most experiments were performed with mice to which the extract had been administered by stomach tube. The usual dose was 10 ml kg^{-1}. In some experiments with mice, and in the one experiment with rats, the extract was administered intraperitoneally.

Antagonism of the antinociceptive effect of morphine

Groups of mice were injected intraperitoneally with morphine sulphate at dose levels of 10 mg kg^{-1} or 20 mg kg^{-1} (hot-plate) or 0·125 mg kg^{-1} (tail-flick). The degree of antinociception was assessed 20 min after injection of the morphine by determining the reaction time after placing the mice on a hot copper surface maintained at 55° (hot-plate; Eddy & Leimbach, 1953) or subjecting the tail to a heat stimulus by laying the tail in a groove 1 mm above an electrically heated wire (tail-flick; Davies, Raventos & Walpole, 1946). In all experiments, except the one in which intraperitoneal and oral administration were compared, the extract was administered by stomach tube 1 h before testing. In one experiment the stability of the solution was assessed by comparing the activity of a freshly re-constituted extract with those re-constituted and left at room temperature and unprotected from light for 1 or 2 weeks.

Production of morphine dependence

Morphine sulphate was administered by twice daily subcutaneous injection. The daily dose was increased from 2 mg kg^{-1} to 400 mg kg^{-1} according to the following schedule

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Dose (mg kg^{-1})	2	4	8	16	32	64	128	256	300	340	380	400	400	400

Five groups of animals were injected according to the above schedule. Extract of *Avena sativa* was administered orally, once daily, at dose levels of 1·0 ml kg^{-1}, and 10 ml kg^{-1} to two groups of mice from day 8–day 14. A further two groups were given the same two doses of extract once only, 1 h before injection of the nalorphine. The extent to which physical dependence had developed was determined by injecting nalorphine (10 mg kg^{-1}) 4 h after the last dose of morphine on Day 14. The number of nalorphine-induced jumps and the number of stools passed was determined during the 10 min period following injection.

Determination of convulsant threshold and barbitone sleeping time

Groups of mice were pretreated with the extract 1 h before intraperitoneal injection of bemegride or nicotine. The convulsant dose 50% was calculated according to the method of Weil (1952). The effect on barbitone sleeping time was determined in mice injected with barbitone sodium (250 mg kg^{-1}) 30 min after the extract.

Determination of pressor response to nicotine

The pressor response to intravenous doses of nicotine was determined in the following groups of urethane (1·5 g kg^{-1}) anaesthetized rats:—(1) control, pretreated with saline only; (2) control to which 5·0 ml kg^{-1} of the extract had been injected intraperitoneally 30 min before the first injection of nicotine; (3) animals to which nicotine had been administered at a dose of 2·5 mg kg^{-1} twice daily for 14–16 days, and (4) animals treated chronically with nicotine as above and to which 5·0 ml kg^{-1} of the extract had been administered intraperitoneally 30 min before the first injection of nicotine. Blood pressure was recorded from the femoral artery using a transducer and a Devices physiological recorder. Nicotine (10, 15 or 20 μg) was injected via a cannula inserted into the femoral vein. 20 min was allowed between successive doses of nicotine.

RESULTS

The antinociceptive effect of morphine was antagonized in both the hot-plate and tail flick tests (Table 1). The antagonism was shown to be dose-related in the hot-plate test (Table 1). Although the extract was administered by stomach tube in nearly all experiments (see Discussion), it was equally effective in its antagonism of morphine when injected intraperitoneally.

Table 1. *Dose-related antagonism of morphine by* Avena sativa, *using hot-plate and tail-flick methods.* The extract was administered by stomach tube 1 h before testing. Reaction time was determined 20 min after i.p. injection of morphine. Figures denote mean ± s.e. with number of observations in parentheses. $*P < 0{\cdot}05$; $**P < 0{\cdot}01$; $***P < 0{\cdot}001$ by Student's *t*-test in comparison with mice treated with morphine alone.

Hot-plate		Tail-flick	
Group	Reaction time (s)	Group	Reaction time (s)
Saline	4·6 ± 0·4 (5)	Saline	3·7 ± 0·3 (5)
Morphine (10 mg kg^{-1})	13·7 ± 1·5 (4)	Morphine (0·125 mg kg^{-1})	11·3 ± 0·9 (6)
Morphine + Dundee '72 Extract (5 ml kg^{-1})	8·0 ± 0·7 (5)**	Morphine + Dundee '72 Extract (10 ml kg^{-1})	8·3 ± 0·8 (6)*
Morphine + Dundee '72 Extract (10 ml kg^{-1})	6·2 ± 0·3 (5)***	Morphine + Extract 33 (10 ml kg^{-1})	8·0 ± 0·5 (6)**
Morphine + Dundee '72 Extract (20 ml kg^{-1})	5·2 ± 0·3 (5)***		

As the extract, diluted with water, was to be used in a trial of potential antismoking agents, it was of obvious importance to ascertain whether the diluted extract retained its potency, albeit as a morphine antagonist in an animal test situation. The extract Dundee '72, reconstituted and left at room temperature unprotected from light for 1 or 2 weeks, was administered at a dose of 10 ml kg^{-1} by stomach tube 1 h before the morphine. Reaction time was tested 20 min after intraperitoneal injection of morphine. Six observations were made in each case. Reaction time on hot-plate (s) was for saline 5·4 ± 0·3, morphine (10 mg kg^{-1}) 14·7 ± 2·1, morphine + 2 week old extract 12·9 ± 2·2, Morphine + 1 week old extract 9·9 ± 1·0 and morphine + freshly prepared extract 8·6 ± 0·9.* ($*P<0{\cdot}02$ by Student's *t*-test in comparison with mice treated with morphine alone.)

A considerable loss of activity from extracts allowed to stand at room temperature and exposed to light for one or two weeks was apparent. Only the freshly prepared extract significantly antagonized the effect of morphine in the hot-plate test, although the one week old extract clearly had some activity.

We next considered the possibility that since the extract exhibited marked antagonism towards the antinociceptive effect of morphine, it may affect the extent of development of physical dependence on morphine when administered together with the morphine. Compared with mice made dependent on morphine alone, the number of nalorphine-induced jumps was approximately halved in animals to which the extract had been administered at a dose level of 10 ml kg^{-1} by stomach tube in addition to the morphine on days 8–14 of the morphine dependence producing programme (Table 2). This difference just failed to reach statistical significance. In mice similarly pretreated with a lower dose of the extract, the number of nalorphine-induced jumps was also reduced, but again the difference did not reach statistical significance. When the extract at the same two dose levels was administered once only, 1 h before injection of nalorphine on day 14, jumping was not elicited nor was the number of jumps induced by nalorphine reduced. The number of stools passed in the 10 min period after injection of nalorphine was reduced in animals to which the extract had been administered once only just before the nalorphine injection or repeatedly on days 8–14 (highest dose only) of the dependence producing program (Table 2).

Table 2. *Effect of* Avena sativa *on the development of morphine dependence.* Morphine dependence was induced by increasing daily s.c. injections of morphine from 2–400 mg kg^{-1}. Jumping was induced in all mice by an i.p. injection of nalorphine 10 mg kg^{-1}. The number of jumps and stools passed were counted during the 10 min period immediately following injection of the nalorphine. High dose extract = 10 ml kg^{-1} (Dundee '72) and low dose extract = 1·0 ml kg^{-1} (Dundee '72). Figures denote mean ± s.e. with number of observations in parentheses. $*P < 0{\cdot}01$, by Student's *t*-test in comparison with the morphine dependent group.

	Number of nalorphine-induced jumps per 10 min (mean ± s.e.)	Number of stools per 10 min (mean ± s.e.)
1. Morphine dependent (14 days)	32·0 ± 6·1 (5)	5·0 ± 0·6 (5)
2. Morphine dependent (14 days) + high dose extract for days 8–14	15·7 ± 5·0 (6)	2·3 ± 0·6 (6)*
3. Morphine dependent (14 days) + low dose extract for days 8–14	19·0 ± 5·0 (4)	2·0 ± 1·1 (4)
4. Morphine dependent (14 days) + high dose extract 1 h before nalorphine	34·0 ± 3·1 (5)	1·4 ± 0·4 (5)*
5. Morphine dependent (14 days) + low dose extract 1 h before nalorphine	32·2 ± 2·1 (5)	2·6 ± 0·6 (5)*

At this stage of our investigations a number of other tinctures of *Avena sativa* became available to us. All had been prepared in the same way as described under Methods and differed only in the part of the plant used, maturity of the plant or geographical location. The relative potency of the various extracts was assessed in terms of their ability to antagonize the analgesic effects of morphine in the hot-plate

test (Table 3). The two locally prepared extracts were found to be the most potent in this respect. An extract prepared from oat seed was found to be of intermediate potency and the extract prepared from brown dry leaves to have very little activity.

That the antagonism of morphine was not secondary to a general increase or decrease in central nervous system excitability was indicated by the finding that the convulsant threshold (CD50) to bemegride (control, 16·2 mg kg^{-1}; extract, 15·4mg kg^{-1}) or nicotine (control 4·04 mg kg^{-1}; extract 4·04 mg kg^{-1}) and the duration of barbitone-induced anaesthesia (control, 1·90 $\pm$ 0·44 (8) h; extract, 1·86 $\pm$ 0·45 (8) h) was not affected by prior administration of the extract at the same dose levels as had previously been found to antagonize the effect of morphine on the hot-plate.

In contrast to the lack of effect of the extract against nicotine-induced convulsions in the mouse, the pressor response to intravenously administered nicotine in the rat

Table 3. ED50 *of various preparations of* Avena sativa *against morphine on the hot-plate.* The extract was administered by stomach tube to groups of 4 mice 1 h before testing on the hot-plate. Morphine 20 mg kg^{-1} i.p. was administered 20 min before testing. The ED50 was determined graphically as the dose of *Avena sativa* required to reduce the antinociceptive effect of morphine by 50%.

Code No.	Type of extract	Source	ED50 (ml kg^{-1})
1	Young green plant	Dundee '73	6·0
2	Young green plant	Dundee '72	7·0
33	Mature plant	Australia	10·8
16	Oat seed		11·5
31	Mature plant	South of England	11·8
17	Young green plant	South of England	15·0
3	Mature green plant	India	15·8
29	Mixture of green and brown leaves only	South of England	17·3
21	Whole mature plant	Greece	19·8
23	Young green plant	South of England	30·0
30	Brown dry leaves only	South of England	35·0 (30% inhibition only)

Table 4. *Effect of* Avena sativa *on the pressor response to nicotine in the anaesthetized rat.* The extract (5·0 ml kg^{-1}, Dundee '72) was injected i.p. 30 min before the first injection of nicotine. Rats were anaesthetized with urethane (1·5 g kg^{-1}). Figures denote mean $\pm$ s.e. with number of observations in parentheses. $*P < 0{\cdot}05$; $**P < 0{\cdot}02$; $***P < 0{\cdot}001$ by Student's *t*-test in comparison with the appropriate control group.

	Intravenous dose of nicotine (μg) 10	15	20	B.p. (mmHg) before first injection of nicotine
Control	19 $\pm$ 2 (10)	29 $\pm$ 3 (10)	38 $\pm$ 3 (14)	78 $\pm$ 3 (7)
Control + extract	15 $\pm$ 1 (11)**	20 $\pm$ 2 (10)**	25 $\pm$ 2 (15)***	97 $\pm$ 7 (6)*
Chronic nicotine (2·5 mg kg^{-1} 2 × daily for 14–16 days)	18 $\pm$ 2 (13)	29 $\pm$ 3 (10)	33 $\pm$ 4 (15)	75 $\pm$ 7 (6)
Chronic nicotine + extract	13 $\pm$ 2 (8)	19 $\pm$ 3 (7)**	34 $\pm$ 5 (10)	81 $\pm$ 10 (4)

was significantly antagonized (Table 4). This was apparent at all three dose levels of nicotine in saline-treated rats and with one of the three doses of nicotine in rats treated chronically with nicotine. The pressor responses to nicotine in rats pretreated with nicotine for 14–16 days were the same as those observed in saline pretreated animals.

DISCUSSION

Although it is not possible to say how tincture of *Avena sativa* first became advocated in the treatment of the "opium habit" (Clarke, 1925) the results of the present experiments provide pharmacological evidence for the presence in the tincture of a substance with morphine antagonistic properties. Thus the extract antagonized the analgesic effect of morphine in two separate tests and, when administered chronically together with morphine, seemed to reduce the extent of development of physical dependence on morphine. However, a single administration of the extract alone did not elicit jumping in morphine-dependent mice nor did it alter the number of jumps induced in these animals by an injection of nalorphine 1 h after the extract. This would suggest that the active principle is not a specific opiate antagonist in the same way as nalorphine or naloxone, although the possibility cannot be excluded that the doses of the extract used in this particular experiment were too low to exert an antagonistic effect. However, the morphine-antagonistic effect would appear to be relatively specific and unrelated to a general central nervous system stimulant or depressant effect, since neither the seizure threshold to chemical convulsant drugs nor the duration of barbitone-induced anaesthesia were affected by prior administration of the extract at dose levels which completely antagonized the analgesic effect of morphine.

Since in the proposed trial of the tincture as a potential anti-smoking agent, the tincture was to be diluted with water and taken orally, it seemed reasonable to investigate the pharmacological properties of the extract in animals after its oral administration. However, in terms of its ability to antagonize the antinociceptive effect of morphine, the activity of the extract appeared to be the same whether administered orally or by intraperitoneal injection. However, the loss of activity from the 2-week old reconstituted extract indicated that any trial with the tincture would best be conducted with the alcoholic tincture diluted with water immediately before ingestion.

Although the extract did not modify the sensitivity to the convulsant effect of nicotine in mice, the pressor effect of intravenously administered nicotine was partially antagonized in the rat. The extract antagonized the pressor response to all three doses of nicotine in previously untreated rats and to one of the three doses in rats pretreated with nicotine for two weeks. Whilst in the first experiment it could be argued that the reduced pressor response was due at least in part to the significantly higher resting blood pressure of the extract-treated rats, there was no such difference between blood pressure of the two groups of rats treated chronically with nicotine. Thus, at least in the rat, *Avena sativa* has been found to antagonize one of the effects of nicotine. It is tempting to speculate that the extract may also antagonize some effects of nicotine in man and that this may be the basis for the observation of Anand (1971) that *Avena sativa* reduced the craving for cigarettes.

It would obviously be desirable to isolate the active principle responsible for the pharmacological effects of *Avena sativa* and to establish whether the same principle is

responsible for both the antagonism of the antinociceptive effect of morphine observed during the present work and the reduction in craving for cigarettes. An alkaloid Avenin (Clarke, 1925), two steroid saponins Avenacosides A and B (Tschesche, Tauscher & others, 1969; Tschesche & Lauven, 1971) and a glycoside Avenacine B (Tschesche, Jha & Wulff, 1973) have been isolated from *Avena sativa*, but whether any of these principles or an as yet unidentified constituent is responsible for the pharmacological activity of the crude extract will have to await further experimentation.

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