Chemical stimulants of shaking behaviour

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A major effort of modern pharmacologic research is directed towards finding chemicals with selective actions on behaviour. Of the drug groups which stimulate specific patterns of movements, the principal, recognized agents are those which cause convulsions, tremors, or increased motor activity and stereotyped behaviour (Goodman & Gilman, 1975). Evidence is presented here for a novel group of chemicals which have, as their predominant effect, the ability to stimulate fur-coated animals to shake like a wet dog. The pharmacodynamic profile of these agents which cause shaking differs from the recognized groups of behavioural stimulants and may represent a new class of drug action on the nervous system.

Shaking as a drug-induced event was first observed in rats undergoing withdrawal from opiates and termed by Wikler as 'wet dog shakes' (Wikler, Green & others, 1960). In certain morphine-dependent animals, the opiate antagonists, nalorphine and naloxone, will precipitate shaking (Grumbach, 1969; Wei, 1973) and, recently, Cowan & MacFarlane (1975) have observed that RX 336M (7,8-dihydro-5′,6′-dimethylcyclohex-5′-eno[1,′2′,8,14]codeinone) a dihydrocodeinone derivative with opiate antagonist activity, causes shaking in non-dependent rats. Another drug which induces shaking is the brain peptide, thyrotrophin-releasing hormone (TRH) (Prange, Breese & others, 1974). Both naloxone in morphine-dependent animals and TRH in normal animals can elicit vigorous shaking at microgram doses injected directly into the medial brainstem (Wei, Siegel & others, 1975a, b). In 1972, Burford & Chappel noted that AG-3-5 (1-[2-hydroxyphenyl]-4-[3-nitrophenyl]-1,2,3,6-tetrahydropyrimidine-2-one), a synthetic chemical being screened for morphine-like analgesic activity, produced in rats a syndrome of 'wet dog shakes', hyperactivity, ptosis and hyperthermia. In this investigation, the first detailed description of AG-3-5 is provided and its pharmacologial properties are compared to naloxone and TRH.

AG-3-5, from A. Foldes and C. Podesva of the Delmar Chemical Co., Montreal, Canada, was dissolved (2 mg ml^{-1}) in hot propylene glycol and administered in variable doses to albino rats (200–300 g), at 4 mg kg^{-1} (i.p.) to hamsters (100–200 g), guinea-pigs (300–400 g), and cats (3–5 kg), at 1 mg kg^{-1} (i.v.) to rabbits (2–2·5 kg), and 0·5 mg kg^{-1} (i.v.) to dogs (15–21 kg). AG-3-5 (2 mg ml^{-1}) was suspended in corn oil when injected at 4 mg kg^{-1} (i.p.) into mice (20–30 g) and gerbils (50–70 g). Shaking, defined as any quick rotational movement of the body around the spinal axis, was counted in alternate 5 min intervals. AG-3-5 was behaviourally active in rats when injected as a suspension in a 30% ethanol–saline solution and when administered by the oral route. Administration of solvents alone did not produce shaking. Male animals were used in all experiments, with at least 4 animals for each dose. Rodents were placed in 1 gallon glass jars for behavioural observations. Rabbits, cats and dogs were observed in the unrestrained state. Detailed dose response and drug interaction studies were carried out mainly in the rat, and for the other species, only the effects of a single dose were studied.

AG-3-5 produced in rats numerous and vigorous 'wet dog shakes' within 2 min after injection. The response was dose-dependent when the number of shakes was summed for a 2 h period (Fig. 1). However, the dose-dependency principally reflected the duration of shaking elicited by AG-3-5 and not the frequency, which generally averaged 4 shakes min^{-1} for all effective doses. Thus, at the lower doses of 0·12 to 1 mg kg^{-1}, shaking lasted approximately 1 h and, at the higher doses of 2 to 8 mg kg^{-1}, for approximately 2 to 3 h. This triggering effect of AG-3-5 on shaking resembles to a certain degree the all or none characteristics of the dose-response relationships in naloxone-precipitated withdrawal shaking (Wei, 1973).

Another behavioural manifestation, obtained mainly with doses of AG-3-5, above 4 mg kg^{-1}, was the attempt of rats to escape from their containers (Wei, Loh & Way, 1973). Of 32 animals tested with doses of 4, 8, or 16 mg kg^{-1} of AG-3-5, 66% of the animals made on the average 21 escape attempts in the 5 to 10 min

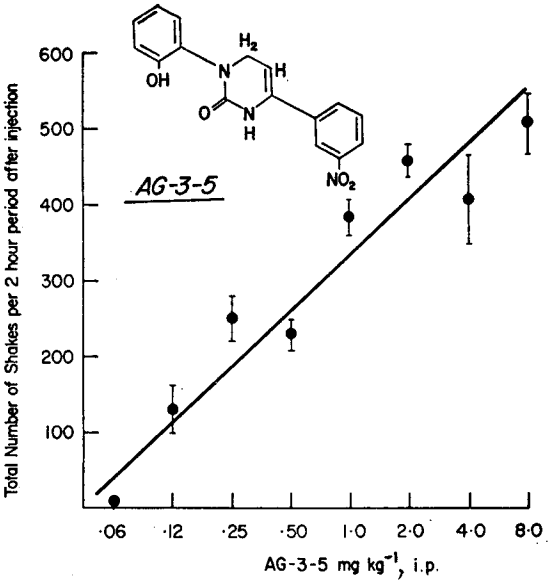

FIG. 1. The dose effect relation of AG–3–5 to shaking.

interval after AG-3-5 injection. In contrast to shaking, however, escape behaviour soon abated and was no longer observed at 20 min after AG-3-5 administration. Body temperature was elevated on the average by 1·5° at 1 h, and 0·5° at 2 h after AG-3-5, 4 mg kg^{-1}. It is not clear if the increase in temperature was a central effect or was secondary to the muscular activity involved in shaking. Body temperature returned to normal 3 h after AG-3-5 when the animal no longer shook.

The effects of central nervous system depressants on AG-3-5-induced shaking were examined. In rats anesthetized with sodium pentobarbitone, 50 (mg kg^{-1}, i.p.), and injected 15 min later with saline or AG-3-5 (8 mg kg^{-1}, i.p.) (n = 10 rats per group), AG-3-5 produced shaking (45 ± 9 shakes *vs* 0 shakes, 30 min observation period after AG-3-5), elevation of body temperature (35·5 ± 0·3° *vs* 33·9 ± 0·4° at 20 min after AG-3-5) and shortening of the duration of barbiturate anaesthesia (74 ± 6 min *vs* 107 ± 7 min). These results were statistically significant ($P < 0.01$, *t*-test). The neuroleptic drugs, haloperidol and perphenazine hydrochloride, attenuated shaking but the most effective drugs in blocking shaking were the opiates, morphine sulphate and (±)-methadone hydrochloride, and the centrally acting sympathomimetic agent, clonidine hydrochloride (Table 1). The opiates and clonidine almost completely suppressed the effects of a 8 mg kg^{-1} (i.p.) dose of AG-3-5.

In all species that were studied AG-3-5 produced shaking and increased locomotor activity. In every animal tested, at least 5 to 10 shakes were observed per animal during the first half hour after AG-3-5. Other signs that appeared included grooming and scratching, which was seen mainly in rats, guinea-pigs, mice and cats; and paw tremor, which was seen mainly in rats and rabbits. In gerbils, guinea-pigs and dogs, AG-3-5 administration made these animals roll on their backs and attempt to rub their fur on the floor. This behaviour, coupled with the shaking, grooming and scratching suggests that AG-3-5 may cause pruritus. Burford & Chappel (1972) have noted that monkeys given AG-3-5 appear preoccupied with skin itchiness.

These results show that AG-3-5, like TRH and certain opiate antagonists, has the unique property of eliciting shaking behaviour in rats. The AG-3-5 induced shaking was blocked by opiates and the central adrenergic agent, clonidine, but barbiturate anaesthesia was less effective. These pharmacological sensitivities also characterize the shaking induced by naloxone-precipitated withdrawal and by TRH, but are not characteristic of behavioural syndromes elicited by other drug classes (Tseng, Loh & Wei, 1975; Wei, 1975). There are, however, sufficient dissimilarities in chemical structure, species specificity and behavioural profile to indicate that AG-3-5, TRH and opiate antagonists do not act on identical pharmacological receptors. Instead, the data show that there are specific neural pathways mediating the shaking response which can be selectively affected by these drugs. In this respect, the actions of AG-3-5 and related drugs may represent and define a new category of drug effects on behaviour.

Table 1. *The effect of centrally acting drugs on shaking induced by AG-3-5 (8 mg kg^{-1} i.p.).* Haloperidol, perphenazine, and morphine sulphate were injected 1 h before AG-3-5. Methadone hydrochloride and clonidine hydrochloride were injected 30 min before AG-3-5. Shaking was counted for 1 h and the results for all drugs are significantly lower than saline controls (n = 8 for each group, $P < 0.01$, Mann-Whitney U test).

Drug	Dose mg kg^{-1}, s.c.	No. shakes in 1 h (mean ± s.e.)
Saline	—	342 ± 28
Haloperidol	2	144 ± 28
Perphenazine	5	100 ± 30
Morphine sulphate	10	18 ± 12
(±)-Methadone HCl	5	0
Clonidine HCl	1	6 ± 2

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Note: U. Jahn & G. Mixich (*Psycopharmacologia*, 1976, **46**, 191–196) observed that certain benzylidene amino oxycarbonic acid derivatives can cause wet dog shakes in normal rats.

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Does cocaine have a post-synaptic action on rat anococcygeus muscle?

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Cocaine is generally considered to potentiate responses to noradrenaline by inhibiting neuronal uptake of the amine. There are, however, numerous examples of potentiation which cannot be wholly explained on this basis (Bevan & Verity, 1967; Reiffenstein, 1968; Kalsner & Nickerson 1969; Maxwell & Eckhardt, 1973; Reiffenstein & Triggle, 1974). In such cases, it has been proposed that cocaine potentiates by acting on the α-adrenoceptors mediating the response (Nakatsu & Reiffenstein, 1968). These authors, using rat vasa deferentia, showed that cocaine caused an increase in the maximum response of tissues which had been treated with an irreversible antagonist (phenoxybenzamine), at a dose sufficient to reduce the maximum response to noradrenaline. As they used equilibrium responses to supramaximal doses of noradrenaline, they concluded that as the proportion of receptors not alkylated by phenoxybenzamine was insufficient to allow a maximum response, the increase in response to noradrenaline caused by cocaine could not be due to a local increase in noradrenaline concentration.

Trendelenburg (1973) has suggested that uptake inhibition is likely to be the mechanism by which cocaine potentiates responses to noradrenaline in tissues with rich noradrenergic innervations, whereas, if potentiation is seen in tissues with sparse noradrenergic innervations, then it is likely to be due to a post-synaptic action.

To test this, we examined the effect of cocaine on responses of isolated rat anococcygeus muscles to supramaximal doses of noradrenaline, both before and after treatment with phenoxybenzamine. This tissue was selected because of its dense noradrenergic innervation.

In each preparation, a log concentration-effect curve to noradrenaline was determined using equilibrium responses, after which the tissue was exposed to phenoxybenzamine at 10^{-8}M or 10^{-7}M for 5 min. The tissue was then washed periodically for 30 min after which responses to an approximate ED50 of noradrenaline were obtained at 5 min intervals until constant responses were obtained (this was never less than 60 min after phenoxybenzamine). Log concentration-effect curves to noradrenaline were then obtained in the absence and presence of cocaine at 4×10^{-6} and 2×10^{-5}M (Fig. 1).

Cocaine shifted the response curves after phenoxybenzamine to the left, indicating either a change in affinity or an increase in the local concentration of noradrenaline. When maximum responses after phenoxybenzamine in the presence of cocaine were compared with the maximum responses after phenoxybenzamine

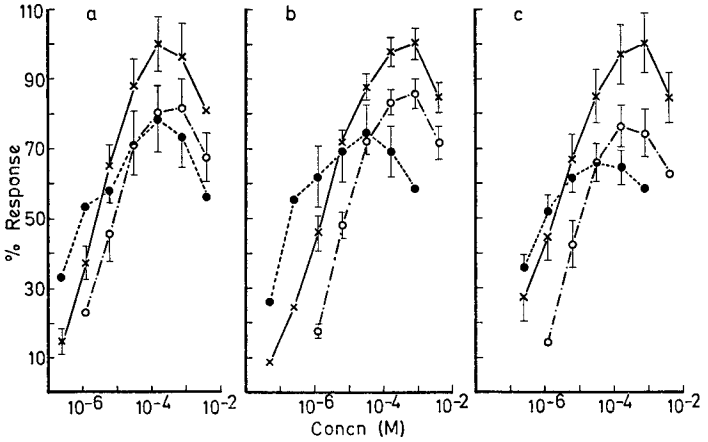

FIG. 1. Effect of cocaine on response of rat anococcygeus muscle to noradrenaline. Mean responses ± s.e.m. expressed as percentages of mean maximal response in each control curve. In no case was the maximum response after phenoxybenzamine in the presence of cocaine significantly greater than the maximal response after phenoxybenzamine in the absence of cocaine. n = 8. S.e.m. bars are omitted when n ≠ 8 (n = 2 to 7). ×——× control, ○—·—·—○ after phenoxybenzamine, ●– – – – ● after phenoxybenzamine in the presence of cocaine. The drug treatments were as follows: Phenoxybenzamine (a) at 10^{-8} M for 5 min, cocaine at 4×10^{-6} M; (b) at 10^{-8} M for 5 min, cocaine at 2×10^{-5} M; (c) at 10^{-7} M for 5 min, cocaine at 2×10^{-5} M.

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