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Effects of *d*-Amphetamine and Apomorphine in a New Animal Model of Petit Mal Epilepsy

G. A. King* and W. M. Burnham

Department of Pharmacology, University of Toronto, Toronto, Ontario M5S 1A8, Canada

Abstract. *d*-Amphetamine is effective in controlling seizures in petit mal epilepsy. The flash-evoked afterdischarge (FEAD) in rats has been proposed as a model of the petit mal seizure. The experiments reported here investigated the dose response relationship for the suppression of FEAD by *d*-amphetamine, and compared its effects with those of the dopamine-mimetic, apomorphine. Significant suppression of FEAD was observed at doses of *d*-amphetamine greater than 0.2 mg/kg. A maximum decrease of 60% occurred at 1.2 mg/kg. Higher doses did not result in any further suppression. In contrast, apomorphine had no effect on the FEAD even at doses that induced intense stereotypic behavior. In other experiments, administration of either the dopamine antagonist pimozide or the α-adrenergic antagonist phenoxybenzamine exacerbated FEAD and also prevented the suppression of FEAD by *d*-amphetamine. The results of these experiments support the hypothesis that the FEAD is a valid model of the petit mal seizure. Furthermore, they provide evidence that norepinephrine is necessary for the seizure-suppressant action of *d*-amphetamine.

Key words: Flash-evoked after discharge – Petit mal seizure – *d*-Amphetamine – Apomorphine

d-Amphetamine is effective in controlling petit mal epilepsy (Strauss 1944; Livingston et al. 1948). It would be useful to study this seizure suppressant effect of amphetamine in a model of the petit mal seizure.

Clonic seizures induced by pentylenetetrazol (PTZ) in rodents have been used extensively as a pharmacological model of the petit mal seizure (Jenny and Pfeiffer 1956; Chen et al. 1963; Swinyard 1972; Krall et al. 1978). Reports of *d*-amphetamine's effect on the PTZ seizure have been contradictory, however, and often inconsistent with clinical data. In mice, for example, *d*-amphetamine has been reported to have no effect on PTZ seizures (Frey 1964), to decrease seizure threshold (Gerald and Riffee 1973; Wolf and Stock 1966; Wolf et al. 1969), to increase seizure threshold (Kilian and Frey 1972), and to both increase and decrease the threshold for clonic PTZ seizures, depending on the dose of *d*-amphetamine (Riffee and Gerald 1976). *d*-Amphetamine also increases the severity of PTZ-induced seizures in rats (Turner and Spencer 1968; Soroko and McKenzie 1970). Therefore, evidence from the PTZ-clonic seizure model does not appear to be consistent with the clinical reports of *d*-amphetamine's effectiveness in controlling petit mal seizures.

A proposed petit mal model, in which amphetamine has given positive results, is the flash-evoked afterdischarge (FEAD). The FEAD is a hypersynchronous burst of repeating slow waves or wave-and-spike complexes, which can be evoked from the visual cortex of the rat (Kimura 1962) and rabbit (Myslobodsky 1976) by presentation of a single light flash stimulus. FEAD is suppressed by drugs that are used to control petit mal seizures, but it is relatively unaffected by drugs like phenytoin, which are used in the treatment of grand mal epilepsy (Shearer et al. 1974; Myslobodsky 1976; King et al. 1980). *d*-Amphetamine suppresses the FEAD in single dose (Fleming et al. 1972; Bigler et al. 1974) and incremental dose (Bigler and Fleming 1976) studies.

In the experiments described here a dose response curve was derived for the effects of *d*-amphetamine on FEAD. The effects of *d*-amphetamine on FEAD have also been compared with those of apomorphine, which directly stimulates dopamine (DA) receptors (Ernst 1967; Anden et al. 1967). Apomorphine shares many of the arousal-inducing effects of amphetamine including behavioral activation (Kelly 1977), EEG desynchronization (Kadzielawa 1974), and decreasing percent time

* *Present address:* Bio-Research Laboratories, 87 Senneville Rd., Senneville, Quebec H9X 3R3, Canada

Offprint requests to: G. A. King

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asleep (Kafi and Gaillard 1976). Investigation of apomorphine's effect on FEAD may provide information on the contribution of DA receptor-activation to seizure suppression by *d*-amphetamine.

A preliminary investigation has been made of the relative contributions of noradrenaline (NE) and DA to the inhibition of the FEAD by *d*-amphetamine through the use of specific receptor blocking drugs. The effects of the α-adrenoceptor blocking drug, phenoxybenzamine, and the DA-antagonist, pimozide, were assessed when applied alone, and when given prior to a maximally effective dose of *d*-amphetamine.

Materials and Methods

Animals and Electrode Implantation

Male, Long-Evans hooded rats (Canadian Breeding Labs, Montreal), 220–260 g, anesthetized with pentobarbital and chloral hydrate were stereotaxically implanted with a stainless steel bipolar electrode (Plastic Products Co., Roanoke, Va, USA, MS 303/3) in the right visual cortex at co-ordinates ant. 2 mm from lambda, and lat. 3.3 mm from the midline (Adams and Forrester 1968). One electrode of the bipolar pair was cut 1 mm longer than the other and this was inserted, under direct visual observation, to a depth of 1 mm in the cortex. Electrical activity was thus recorded transcortically between the electrode at the cortical surface and the electrode 1 mm beneath the surface. The bipolar electrode was fixed to the skull with dental cement anchored by four jewellers' screws. One screw also served as a ground connection. Following surgery the wound was sutured and the animal was returned to its cage for at least 1 week prior to the start of habituation.

Rats used for experiments with pimozide and phenoxybenzamine had been used previously in similar experiments, but were drug-free for at least 1 month prior to the commencement of testing. All other animals were naive.

Apparatus

The test apparatus consisted of boxes (14.3 cm wide / 19.7 cm deep / 30.5 cm high), whose front and top were of clear Plexiglas, while the sides and floor were painted white. The test boxes were located inside a sound attenuating chamber (86.4 cm wide / 73.7 cm deep / 81.3 cm high) painted white inside. Flash stimuli generated by a Grass PS-2 stroboscope (intensity setting 16) were reflected from the ceiling of the sound attenuating chamber. The stroboscope was triggered by a Grass S-48 stimulator. No ambient lighting was provided in the chamber, and white noise was present during testing in order to mask extraneous noise and the click associated with operation of the flash. The EEG and the marker pulse from the stroboscope were recorded on paper on a Grass Model 8–10 electroencephalograph. The superficial pole of the transcortical bipolar electrode was always connected to the GI amplifier input.

Habituation and Test Procedure

The same test procedure was used during both habitution and drug testing. Habituation to the test procedure commenced 1 week (minimum) following surgery. Awake, freely moving animals were exposed to the test conditions once each day for 7 days prior to the start of drug testing. Mydriasis was induced by the application of a 2% solution of homatropine. Each animal was then placed in a test box, electrode leads connected, and light flash stimulation presented at 1 per 4 s for 8 min (120 stimulus presentations). Parametric studies have demonstrated that, under similar conditions FEAD responding stabilizes after 3 days (Bigler et al. 1976).

Drug Treatments

a) d-Amphetamine and Apomorphine. A total of 12 rats with satisfactory EEG's and a stable response to the flash stimulus were chosen and divided into two groups of six. Each group was tested with five doses of either *d*-amphetamine or apomorphine plus one saline control injection. Successive drug tests were separated by 10-day intervals. The order in which different doses of a drug were administered was determined for each group by means of a Latin square. *d*-Amphetamine was injected 30 min and apomorphine 15 min prior to testing.

b) Drug Interaction Experiments. The effects of phenoxybenzamine and pimozide alone, and in combination with *d*-amphetamine were measured in four other groups of seven animals each. These animals, who had been previously exposed to the FEAD paradigm, were given four habituation sessions prior to drug testing.

Two groups were tested first with the appropriate vehicle and then 10 days later with either 10 mg/kg of phenoxybenzamine or 1.2 mg/kg of pimozide. The other two groups were tested with three successive drug combinations: vehicle + saline; vehicle + *d*-amphetamine (1.2 mg/kg); and pimozide (1.2 mg/kg) or phenoxybenzamine (10 mg kg) + *d*-amphetamine (1.2 mg/kg). This dose of phenoxybenzamine causes clear-cut postsynaptic α-adrenoceptor blockade in rats without antagonizing the effects of dopamine receptor stimulation (Anden and Strombom 1974). The dose of pimozide chosen antagonizes the behavioral effects of 10 mg/kg of *d*-amphetamine without blocking α-adrenergic activity (Janssen et al. 1968). *d*-Amphetamine was injected $^1/_2$ h, phenoxybenzamine 1 h, and pimozide 4 h prior to testing. Ten days elapsed between successive drug treatments. Test conditions were identical to those described above.

d-Amphetamine (Novopharm Scarborough, Ont., Canada) and apomorphine (Ayerst, Montreal, Que., Canada), were dissolved in saline. Phenoxybenzamine (Smith Kline and French, Mississauga, Ont., Canada) was dissolved in saline with a few drops of 1 N HCl. Pimozide (McNeill, Stouffville, Ont., Canada) was dissolved in 0.1 M tartaric acid. All drugs were injected IP in a volume of 0.1 ml/100 g.

Data Analysis

For all animals in all groups, records were scored by measuring the total duration of FEAD for 120 stimulus presentations. The initial oscillations in the EEG following a flash, including the first large amplitude slow wave, were considered to be the normally occurring flash-evoked potential ('FEP', see Fig. 1a) and were not included in measurements of the FEAD. The succeeding waves or wave-and-spike complexes were considered to be the FEAD (Fig. 1b). In order for EEG oscillations following the flash-evoked potential (FEP) to be included as FEAD they had to consist of two or more waves or wave-and-spike complexes with an average intraburst frequency of ten per second or less.

The total FEAD duration at each drug dose was expressed as a percentage of that following injection of vehicle. Analysis of variance was performed for each drug group on log transforms of the FEAD scores.

Results

d-Amphetamine (0.2–7.5 mg/kg) suppressed the average amount of FEAD below saline control levels

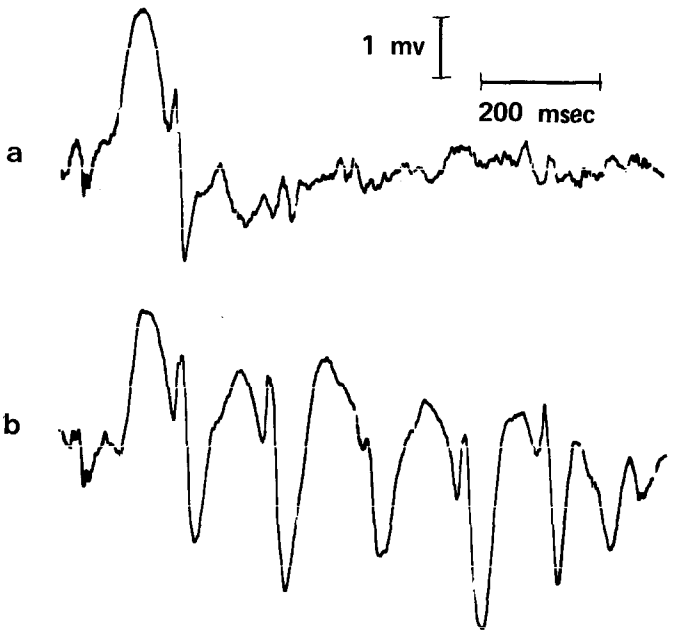

Fig. 1a and b
Oscilloscope traces of one s of EEG activity, triggered by light flash stimuli. **a** FEP with a total duration of approximately 250 ms, followed by a return to desynchronized EEG activity. **b** FEP, as in **a**, this time precedes an afterdischarge (FEAD)

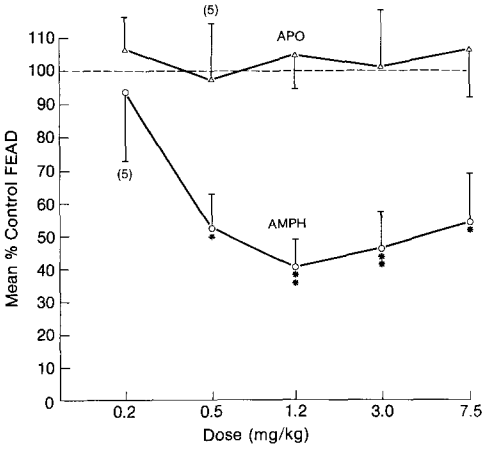

Fig. 2. Dose response curves for the effects of *d*-amphetamine and apomorphine on the amount of FEAD. The ordinate indicates the mean amount of FEAD expressed as a percent of saline control. Each point is the average of data from six rats, bars represent the SEM. (5) designates points for which data from one animal was lost due to equipment failure. Difference between treatment means and control (100%) analyzed by Dunnett's *t*-test (Winer 1962). * $P < 0.025$; ** $P < 0.005$

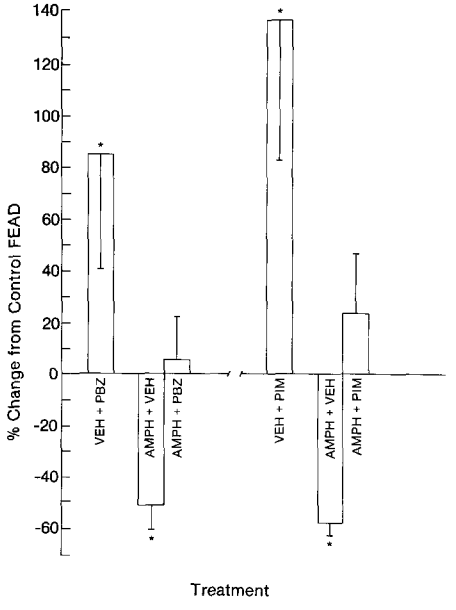

Fig. 3. Bar graph of the effects on FEAD of phenoxybenzamine (PBZ, 10 mg/kg), pimozide (PIM, 1.2 mg/kg), and *d*-amphetamine (AMPH, 1.2 mg/kg) combined with the appropriate vehicle (VEH); and of the effects of AMPH given after injection of PBZ or PIM. Mean total FEAD duration expressed as percent change from control FEAD. $N = 7$ in all cases, lines represent SEM. Comparisons between treatment means and control by Dunnett's *t*-test (Winer 1962). * $P < 0.025$

($F = 5.07$, df 5,24, $P < 0.01$). FEAD was reduced by 60% at 1.2 mg/kg and higher doses did not induce any further decrease (Fig. 2). Apomorphine, on the other hand, did not affect FEAD at any dose (Fig. 2). No significant treatment effect was found for the apomorphine group ($F = 0.42$, $df = 5,23$).

Apomorphine and amphetamine both induced stereotypic behavior in all animals, which was observable at the time of testing. The intensity of the stereotypic response to both drugs appeared to be dose-related.

Both pimozide and phenoxybenzamine given alone increased the average amount of FEAD (Fig. 3). When

injected prior to *d*-amphetamine, both phenoxybenzamine and pimozide prevented the suppression of FEAD (Fig. 3).

Discussion

The experiments presented here examined the effects of *d*-amphetamine on FEAD over a wide range of drug doses. Furthermore, dopaminergic and noradrenergic involvement in the suppression of FEAD by *d*-amphetamine were investigated by comparing the effects of *d*-amphetamine with those of the DA-agonist apomorphine, and by attempting to reverse the effects of *d*-amphetamine with drugs that are specific antagonists for either dopaminergic or noradrenergic receptors.

d-Amphetamine caused significant suppression of FEAD at doses between 0.5 mg/kg and 7.5 mg/kg. These results are consistent with FEAD's proposed role as a model of petit mal epilepsy (Shearer et al. 1974; Myslobodsky 1976; King et al. 1980) and with reports of the beneficial therapeutic effect of amphetamine in the treatment of petit mal epilepsy (Strauss 1944; Livingston 1948). In contrast, the widely used PTZ-clonic seizure model has yielded conflicting results concerning the seizure-suppressant effects of *d*-amphetamine (see Introduction). The results of the present experiments and those of previous studies (Fleming et al. 1972; Bigler et al. 1974; Bigler and Fleming 1976) suggest that FEAD is superior to the PTZ-clonic seizure model for screening amphetamine-like drugs and for investigating their mechanism of action.

Unlike *d*-amphetamine, apomorphine had no effect on the amount of FEAD, even at high doses that induced marked stereotypic behavior. Apomorphine stimulates DA receptors directly (Ernst 1967; Anden et al. 1967), and increases behavioral activity in a manner similar to *d*-amphetamine (Kelly 1977). Apomorphine also increases the percent time awake (Kafi and Gaillard 1976) and induces desynchronized EEG activity (Kadzielawa 1974). These arousal effects are similar to those induced by amphetamine (Rechtschaffen and Marion 1964; Bradley and Elkes 1957). Despite these similarities, the direct activation of DA receptors by apomorphine does not appear to be sufficient to inhibit FEAD.

The results of experiments with specific receptor blockers appear to implicate both NE and DA in the seizure suppressant effects of *d*-amphetamine. Both phenoxybenzamine and pimozide given alone increased the amount of FEAD, and both drugs prevented the suppression of FEAD when given prior to *d*-amphetamine. These results could be interpreted to mean that the FEAD-suppressant effects of amphetamine result from interactions with both DA and NE. However, this conclusion is not supported by the negative results obtained with apomorphine.

The lack of congruence between the results obtained with apomorphine and those seen with pimozide might be explained by the proposed existence of multiple, functionally distinct types of DA-receptors, which repsond differently to apomorphine and DA (Cools and van Rossum 1976; Kebabian and Calne 1979). Cools and van Rossum (1976) have proposed the existence of two DA receptors mediating excitation and inhibition respectively. DA is thought to act as an agonist at both receptors, while apomorphine is effective only at the excitatory receptor (Cools and van Rossum 1976). Two functionally distinct DA-receptor types have also been proposed by Kebabian and Calne (1979), who suggest that one receptor activates adenylate cyclase, while the other receptor is not enzyme-linked. DA is proposed to act as an agonist at both receptors, but apomorphine is a full agonist only at the non-enzyme-linked receptor (Kebabian and Calne 1979). If either of these proposed systems is present, then endogenously released DA would be expected to produce some effects that could not be mimicked by apomorphine.

The results presented here support the hypothesis that FEAD is a valid model of the petit mal seizure, and that it may be especially useful for investigating the mechanism of action of amphetamine and amphetamine-like drugs in the control of petit mal epilepsy. The results of preliminary experiments designed to investigate the role of DA and NE in amphetamine's mechanism of action more strongly suggest a role for NE than for DA. Other experimental approaches, especially the use of discrete neurochemical lesioning techniques, are needed in order to investigate further the contributions of NE and DA to the antiepileptic effects of amphetamine.

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