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BRIEF COMMUNICATION

Action of Fenfluramine, Phenylpropanolamine, Phentermine and Diethylpropion on Acoustic Startle in Rats[1]

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KUTSCHER, C. L. *Action of fenfluramine, phenylpropanolamine, phentermine and diethylpropion on acoustic startle in rats*. PHARMACOL BIOCHEM BEHAV 27(4) 749–752, 1987.—Four commonly used anorectics which are amphetamine analogues were tested for their action on responsiveness in an acoustic startle test when rats were given daily IP injections adequate to produce a change in body weight. Drugs were given for 22 days. None of these drugs increased startle responsiveness as does the amphetamine parent compound. Instead, fenfluramine and phenylpropanolamine decreased startle responsiveness and phentermine and diethylpropion produced no change. There was no relationship between drug action and body weight. Partial tolerance was found for the fenfluramine action on startle and complete tolerance was found for its action on body weight gain. The fenfluramine action is compatible with the extensive literature on humans and animals indicating sedative properties.

Anorectics Diethylpropion Fenfluramine Phentermine Phenylpropanolamine

THE neurobehavioral actions of amphetamine have been well studied and are considered to be rather well understood [6,12]. Amphetamine is composed of the phenylethylamine skeleton and the substitution of a methyl group at the alpha carbon. Other substitutions on this skeleton have produced a family of analogues with anorectic properties which are widely used for weight control, e.g., phentermine (PTM), fenfluramine (FEN), diethylpropion (DEP) and phenylpropanolamine (PPA) [18]. Although action of these drugs on food intake has been intensively studied [7] other neurobehavioral actions have been studied in less detail.

In this experiment, the action of isomolar doses of these anorectics sufficient to produce weight loss (except in the case of phenylpropanolamine) was studied in regard to effect on acoustically-induced startle response. Previous studies have shown that the latency of the startle response is only about 8 msec suggesting that the neural circuitry which mediates the response, presumably located in the midbrain, must contain only a few synapses [3]. Action of these phenylethylamine-derived anorectics on startle has not been studied. This test may tap one useful and easily accessible dimension of action of anorectics on the CNS.

METHOD

Animals

Naive, male Long-Evans hooded rats, 80–87 days old were used in this experiment. They were bred in the Behavioral Neuroscience Laboratory at Syracuse University. Since our male rats gain weight steadily for long periods, the use of males permits observation of two possible actions of the anorectics—reduction in weight or reduction in rate of weight gain.

Drugs

Drugs were obtained from Sigma (St. Louis, MO). All drugs were dissolved in 0.9% NaCl vehicle and were injected IP in an injection volume of 1 ml/kg. Drugs and dosages were: PPA, 7.08 mg/kg; FEN, 10.12 mg/kg; DEP, 9.12 mg/kg; PTM, 7.0 mg/kg; and the saline vehicle (SAL).

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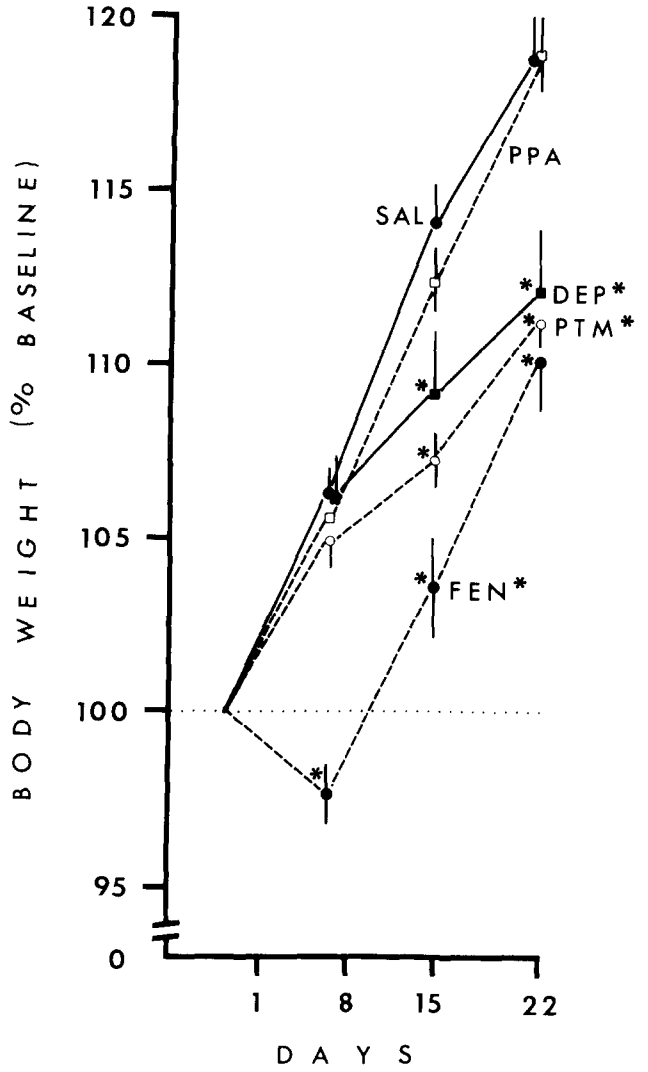

FIG. 1. Body weights as percentage of preinjection weights recorded immediately before injection of drugs on days when startle was measured. Group designations are: SAL, saline; PPA, phenylpropanolamine; DEP, diethylpropion; PTM, phentermine; FEN, fenfluramine.

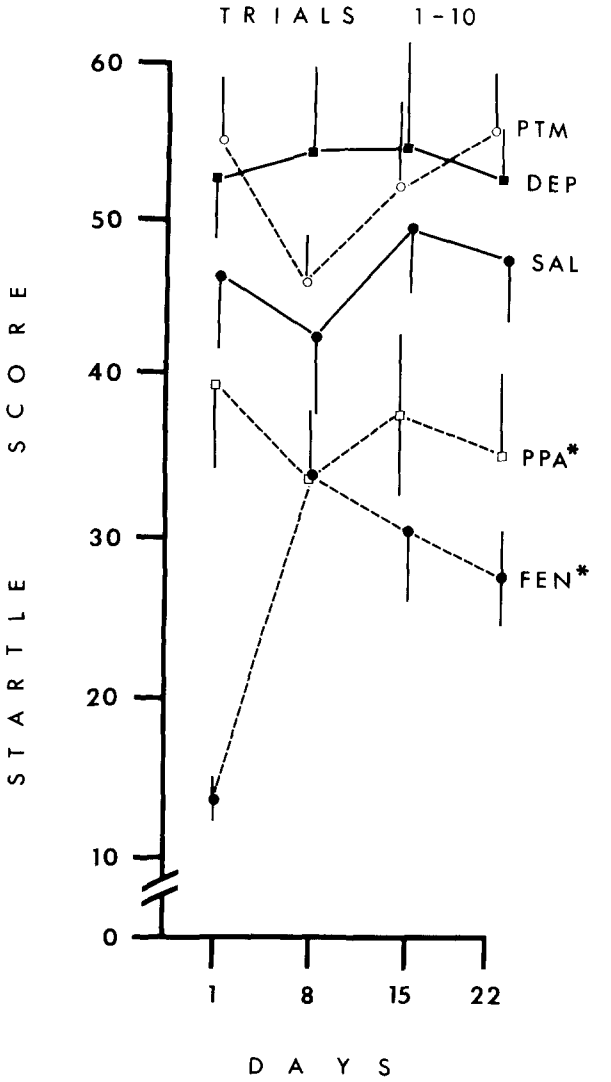

FIG. 2. Mean startle response on first ten trials.

Startle Measurement

Chambers of measurement of acoustic startle were 10×20×11 cm, constructed of 1.3 cm mesh hardware cloth covered with fine mesh screen. Chambers rested on springs with lateral movement restricted by posts within the springs which also passed through holes in the platform holding the test cage. An accelerometer on the bottom of each cage converted cage movement into a voltage change which was digitized by an A-D converter and recorded by a computer. The maximum voltage change during the 50 msec following the initiation of the tone was recorded as the measure of startle. The tone was 120 dB, 4000 Hz, delivered for 50 msec for 50 trials. The intertrial interval was 8 sec. The trial sequence was begun 5 min after rats were placed into the test cages. A white noise background tone of 60 dB was on continuously in the test room which was 160×168×196 cm with walls padded with sound-attenuating material (Styrofoam).

Procedure

Fifteen rats were tested in each of the drug groups. The experiment was conducted in three replications. At the beginning of each replication rats were removed from the breeding colony and housed in a holding room, 5–6/cage in stainless steel cages, 63×25×18 cm. Tap water and Purina Chow were available continuously. On the day before the first injection, rats were gentled by holding them and stroking them for one 1 min. All injections and startle tests were conducted in the afternoon.

Rats were weighed and injected daily for 22 consecutive days. On Days 1, 8, 15 and 22 they were also tested in the auditory startle apparatus 15 min after the injection. On those days rats were weighed and removed from the holding room and placed into individual plastic cages 28×17×13 cm on a cart outside the startle-testing room. They were removed briefly from these cages for the injection.

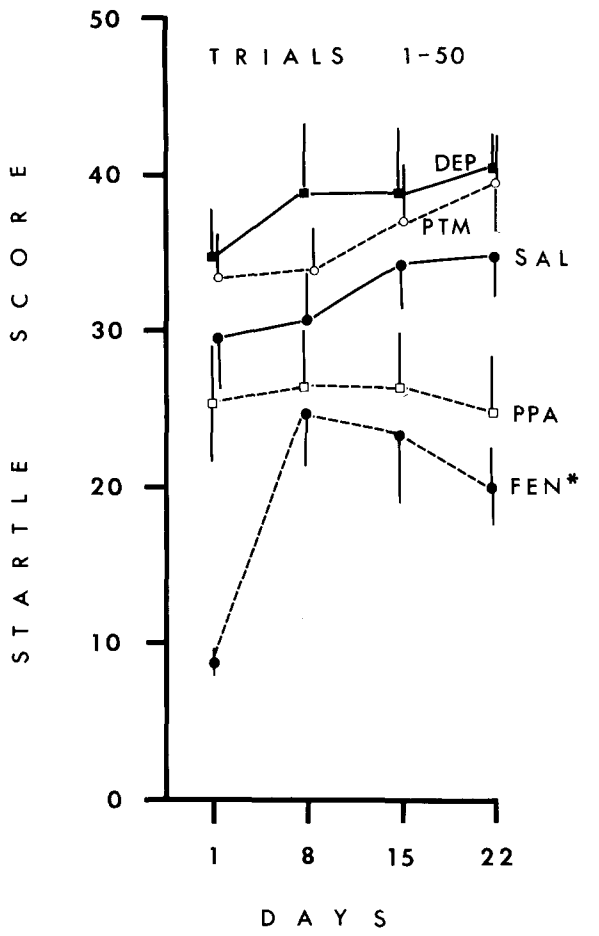

FIG. 3. Mean startle response on all 50 trials.

On days when startle was not tested, rats were removed from the home cage, weighed, injected and returned immediately to the group home cage.

RESULTS

Body Weight

Weights on Days 8, 15 and 22 were converted to a percentage of weight at the time of the first injection on Day 1 (baseline) and are shown in Fig. 1. Weights and startle scores were analyzed with a split-plot analysis of variance to determine the effect of days of injection, drug and the interaction between the two. Post hoc comparisons of individual means were done with the Newman-Keuls test with the alpha level set at 0.05. In some cases simple main effects tests were run to interpret interactions [11].

Weights differed as a function of drug type, $F(4,70)=12.94$, $p<0.001$, and days of injection, $F(2,140)=261.55$, $p<0.001$. The drug × day interaction was also significant, $F(8,140)=7.71$, $p<0.001$. Post hoc comparisons of drug groups, collapsing over injection days, showed that PPA had no effect on body weight, but FEN, PTM and DEP all produced lower weights than the SAL control group. Simple main effects tests run within each day showed significant drug action on Day 8, $F(4,70)=13.41$, $p<0.001$, Day 15, $F(4,70)=11.35$, $p<0.001$, and Day 22, $F(4,70)=11.06$, $p<0.001$. Post hoc tests showed that on Day 8 only FEN-injected animals differed from the SAL group. On Days 15 and 22, FEN, DEP and PTM all differed from SAL, but did not differ from each other.

Startle Score Trials 1–10

Figure 2 shows the mean startle responses calculated over the first 10 trials only. Scores differed as a function of drugs, $F(4,70)=11.02$, $p<0.0001$, but not as a function of injection day, $F(3,210)=1.10$, $p<0.35$, however, there was a significant interaction, $F(12,210)=2.35$, $p<0.008$. A post hoc test, collapsing over days, revealed that both FEN and PPA depressed startle relative to the SAL control group and the PTM and the DEP groups. Simple main effects testing within each drug group indicated that only in the FEN group was startle influenced by day of injection, $F(4,56)=6.91$, $p<0.001$. Post hoc testing within the FEN group showed that startle scores were lower on Day 1 than on the other three days which did not differ from each other.

Startle Scores Trials 1–50

These startle scores (Fig. 3) were similar in pattern of drug action to those calculating only on the first 10 trials. Scores differed as a function of drug, $F(4,70)=7.69$, $p<0.001$, day of injection, $F(3,210)=6.84$, $p<0.001$. The interaction was also significant, $F(3,210)=2.07$, $p<0.02$. Post hoc testing, collapsing over test days, showed a significant depression of FEN-treated rats, but not for PPA-treated rats, compared to the SAL-treated rats, however, PPA-treated rats were significantly less responsive in startle than those given isomolar doses of PTM or DEP.

DISCUSSION

In this study none of these phenylethalamine analogues reliably increased startle responsiveness as is the expected amphetamine action [2,10]. The trends for PTM and DEP were not significant in any of the analyses even though dosages of these two compounds were sufficient to produce a significant, although delayed, reduction in rate of weight gain. By contrast FEN produced only a transient change in body weight followed by weight gain at approximately the same rate as the SAL group and the PPA group (which showed no weight change). Both FEN and PPA produced decreased startle responsiveness, however the FEN action was more robust than that of PPA which did not appear in both analyses. In either case startle responsiveness did not relate to weight change; PPA-treated rats did not show weight change and FEN-treated rats showed the greatest depression of startle immediately after the first injection on Day 1 before any weight loss had occurred.

The reduced startle responsiveness produced by FEN was not accompanied by ptosis or limb rigidity, even on Day 1. Although a peripheral motor deficit cannot be ruled out without additional work, the extensive literature on FEN contains the basis for postulating a CNS mechanism. FEN's actions on serotonergic neurons include release of neurotransmitter, blockade of reuptake and direct stimulation of receptors [14]. An extensive review on the pharmacology of startle [3] indicates that drugs which facilitate serotonergic transmission decrease startle responsiveness. FEN-induced anorexia [13] and stereotypy [17] are also serotonergically mediated. Side effects in humans taking FEN for weight con-

trol are reduced energy, lethargy, drowsiness and tiredness [8,16]—i.e., depression, not stimulation.

The possibility exists, therefore, that the greatly reduced startle score following the first FEN injection could result from enhanced serotonin action. The partial tolerance to FEN with chronic administration could result from a long-term depletion of serotonin [5] possibly produced by a reduction in serotonin synthesis [9].

The physiological basis of PPA's action on startle is unclear given the surprising paucity of reports on the CNS action of this widely-used drug which is often sold as an amphetamine look-alike or legal stimulant [1]. PPA produced inhibition of Type B MAO in both human and animal tissue [20] and dopamine depletion in rats in very high doses [19], but neither action suggests a basis for depression of startle.

DEP and PTM, in contrast to FEN, produce anorectic actions by means of catecholaminergic neurons similar to the mode of action of amphetamine [15]. In humans, chronic administration for weight control produced side effects which were infrequent and mild [4].

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REFERENCES

1. Bernstein, E. and B. M. Diskant. Phenylpropanolamine: A potentially hazardous drug. *Ann Emerg Med* **11:** 311–315, 1982.
2. Davis, M., T. H. Svensson and G. K. Aghajanian. Effects of d- and l-amphetamine on habituation and sensitization of the acoustic startle response in rats. *Psychopharmacologia* **43:** 1–11, 1975.
3. Davis, M. The mammalian startle response. In: *Neural Mechanisms of Startle,* edited by R. C. Eaton. New York: Plenum, 1984, pp. 287–352.
4. Douglas, A., J. G. Douglas, C. E. Robertson and J. F. Munro. Plasma phentermine levels, weight loss and side-effects. *Int J Obes* **7:** 591–595, 1983.
5. Ghezzi, D., R. Samanin, S. Bernasconi, G. Togoni, M. Gerna and S. Garattini. Effect of thymoleptics on fenfluramine-induced depletion of brain serotonin in rats. *Eur J Pharmacol* **24:** 205–210, 1973.
6. Groves, P. M. and G. V. Rebec. Biochemistry and behavior: Some central actions of amphetamine and antipsychotic drugs. *Annu Rev Psychol* **27:** 91–127, 1976.
7. Hoebel, B. The psychopharmacology of feeding. In: *Handbook of Psychopharmacology, Vol 8: Drugs, Neurotransmitters and Behavior,* edited by L. L. Iversen, S. D. Iversen and S. H. Snyder, New York: Plenum, 1977, pp. 55–129.
8. Innes, J. A., M. L. Watson, M. J. Ford, J. F. Munro, M. E. Stoddart and D. B. Campbell. Plasma fenfluramine levels, weight loss and side effects. *Br Med J* **2:** 1322–1325, 1977.
9. Invernizzi, R., C. Berettera, S. Garattini and R. Samanin. D- and l-isomers of fenfluramine differ markedly in their interaction with brain serotonin and catecholamines in the rat. *Eur J Pharmacol* **120:** 9–15, 1986.
10. Kehne, J. H. and C. A. Sorenson. The effects of pimozide and phenoxybenzamine pretreatments on amphetamine and apomorphine potentiation of the acoustic startle response in rats. *Psychopharmacology (Berlin)* **58:** 137–144, 1978.
11. Kirk, R. E. *Experimental Design,* 2nd edition. Belmont, CA: Brooks/Cole, 1982.
12. Kokkinidis, L. and H. Anisman. Amphetamine models of paranoid schizophrenia: An overview and elaboration of animal experimentation. *Psychol Bull* **88:** 551–579, 1980.
13. Mennini, T., A. De Blasi, E. Borroni, C. Bendotti, F. Borsini, R. Samanin and S. Garattini. Biochemical and functional studies on tolerance to anorectic activity of d-fenfluramine in comparison to amphetamine. In: *Anorectic Agents: Mechanisms of Action and Tolerance,* edited by S. Garattini and R. Samanin. New York: Raven Press, 1981, pp. 87–100.
14. Mennini, T., S. Garattini and S. Caccia. Anorectic effect of fenfluramine isomers and metabolites: Relationship between brain levels and in vitro potencies on serotonergic mechanisms. *Psychopharmacology (Berlin)* **85:** 111–114, 1985.
15. Samanin, R., S. Bernasconi and S. Garattini. The effect of selective lesioning of brain catecholamine-containing neurons on the activity of various anorectics in the rat. *Eur J Pharmacol* **34:** 373–375, 1975.
16. Riley, I., J. Corson, I. Haider and I. Oswald. Fenfluramine overdose. *Lancet* **2:** 1162–1163, 1969.
17. Trulson, M. E. and B. L. Jacobs. Behavioral evidence for the rapid release of CNS serotonin by PCA and fenfluramine. *Eur J Pharmacol* **36:** 149–154, 1976.
18. Weiner, N. Norepinephrine, epinephrine, and the sympathomimetic drugs. In: *The Pharmacological Basis of Therapeutics,* edited by A. Gilman, L. Goodman, T. Rall and F. Murad. New York: Macmillan, 1985, pp. 145–180.
19. Woolverton, W. L., C. E. Johanson, R. De-la-Garza, S. Ellis, L. S. Seiden and C. R. Schuster. Behavioral and neurochemical evaluation of phenylpropanolamine. *J Pharmacol Exp Ther* **237:** 926–930, 1986.
20. Yu, P. H. Inhibition of monoamine oxidase activity by phenylpropanolamine, an anorectic agent. *Res Commun Chem Pathol Pharmacol* **51:** 163–171, 1986.