Pharmacology Biochemistry & Behavior, Vol. 26, pp. 119–121, 1987. © Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

Cholinergic Involvement in the Action of Formetanate on Operant Behavior in Rats[1,2]

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Received 27 June 1986

MOSER, V. C. AND R. C. MACPHAIL. *Cholinergic involvement in the action of formetanate on operant behavior in rats.* PHARMACOL BIOCHEM BEHAV **26**(1) 119–121, 1987.—Formetanate (FMT) is a formamidine acaricide/insecticide with a carbamate moiety in its molecular structure. FMT-induced lethality is reportedly due to inhibition of acetylcholinesterase. Here we report evidence of the neurochemical basis for the sublethal, behavioral effects of FMT in rats. In this experiment, 0.5 mg/kg of FMT (5 min before the 55-min test session) produced a pronounced suppression of response rates in rats trained to lever-press under a multiple fixed-interval 1-min fixed-interval 5-min schedule of milk reinforcement. Injections of scopolamine (0.1 mg/kg) and methylscopolamine (0.1 mg/kg) 15 min before FMT blocked the response rate suppression, whereas pretreatment with either mecamylamine (2 mg/kg) or hexamethonium (2 mg/kg) did not. These data suggest that FMT acts as an indirect agonist on central and peripheral muscarinic receptors, by inhibiting acetylcholinesterase, to produce changes in schedule-controlled responding.

Formetanate Formamidine Carbamate Cholinergic antagonists Schedule-controlled performance Rats

FORMETANATE (FMT) has been used worldwide as an insecticide/acaricide for more than a decade. Due to its structural configuration, it has been classified as both a formamidine and a carbamate pesticide (see Fig. 1). Knowles and Ahmad [4] demonstrated a correlation between the onset and duration of acetylcholinesterase (AChE) inhibition in rat brain, concentration of FMT in brain, and signs of intoxication such as tremor and convulsions following a lethal dose of FMT. We have shown [6] that FMT is a more potent inhibitor of AChE than of monoamine oxidase (MAO), an action often ascribed to formamidines. For purposes of further understanding FMT intoxication, it was important to determine whether the behavioral effects produced by sublethal exposures were more formamidine- or carbamate-like. Therefore, in this experiment we investigated the importance of cholinergic involvement in the effects of FMT on schedule-controlled operant behavior in rats.

METHOD

Animals

Seven adult male Long-Evans hooded rats (Charles River Co.) were maintained at 350 g b.wt. via restricted daily food intake. The rats had previously been used to determine the acute effects of several formamidine pesticides on schedule-controlled behavior [7]. Water was available ad lib in the home cages, but not during experimental sessions or in the transport cages.

Apparatus

Sessions took place in commercial operant chambers (Coulbourn Instruments Co., Model E10-10) located inside larger ventilated, sound- and light-attenuating chambers (Gerbrands Corp., G7211). A response lever was located on

[1]We gratefully acknowledge the expert technical assistance of Mr. K. E. Wiggins in the conduct of this research. This experiment was reported at the 15th annual Society for Neuroscience meeting, Dallas, TX (*Soc Neurosci Abstr* **11**: 1293, 1985).

[2]This manuscript has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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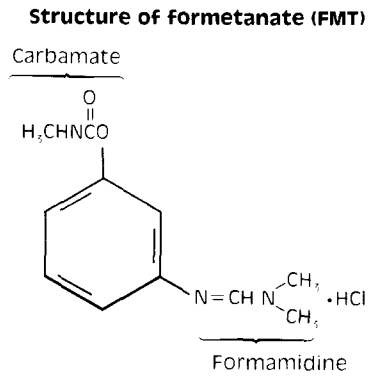

FIG. 1. Structure of formetanate (FMT).

the left side of the front panel, 2.5 cm above the chamber floor, and a recessed dipper magazine was on the right. Each chamber also contained three cue lights located directly above the response lever, a centrally located houselight on the front panel 27 cm above the floor, and a light above the dipper trough. The dipper delivered approximately 0.05 ml of milk (one part Borden's Eagle Brand sweetened condensed milk:two parts water). Schedule contingencies and data collection were controlled by a superSKED software system (State Systems, Inc.) with a PDP8/A minicomputer (Digital Equipment Corp.).

Procedure

Each rat was previously trained to press a lever under a multiple fixed-interval (FI) 1-min FI 5-min schedule of milk reinforcement [2] during daily 55-min sessions (Monday–Friday). A 30-sec limited hold was in effect at the end of each interval. Thus, according to this schedule the first response occurring after either 1 or 5 min, but before 1.5 or 5.5 min, was reinforced. A 10-sec time-out (no lights on) followed the delivery of each reinforcer or the limited hold, during which time responses had no programmed consequences. The schedule components and their respective stimuli (cue lights on for FI 5-min and the houselight on for FI 1-min) alternated throughout the session after each time-out period. These lights were extinguished upon delivery of the reinforcer, and the dipper light was illuminated for the 4 sec that milk was available.

Dosing occurred ordinarily on Tuesdays and Fridays, and the data collected on Thursdays served as control values for evaluating treatment effects. A dose of FMT HCl (0.5 mg/kg) which was previously shown [7] to substantially suppress responding when given alone, was given 5 min before the session in combination with either saline or one of several cholinergic antagonists, which were given 20 min pre-session. The antagonists included scopolamine HBr and methylscopolamine Br (0.1 mg/kg each), and mecamylamine HCl and hexamethonium HBr (2 mg/kg each). Dosages were selected on the basis of preliminary determinations. Scopolamine and mecamylamine produce muscarinic and nicotinic blockade, respectively, in both the central and peripheral nervous system [10,12]. Methylscopolamine and hexamethonium produce muscarinic and nicotinic blockade, respectively, that is restricted largely to the peripheral nervous system [10,12]. The effect of each antagonist was also determined when given in combination with saline (5 min pre-session). FMT dosing was always separated by at least 5 days in an attempt to prevent the development of tolerance. All injections were administered intraperitoneally.

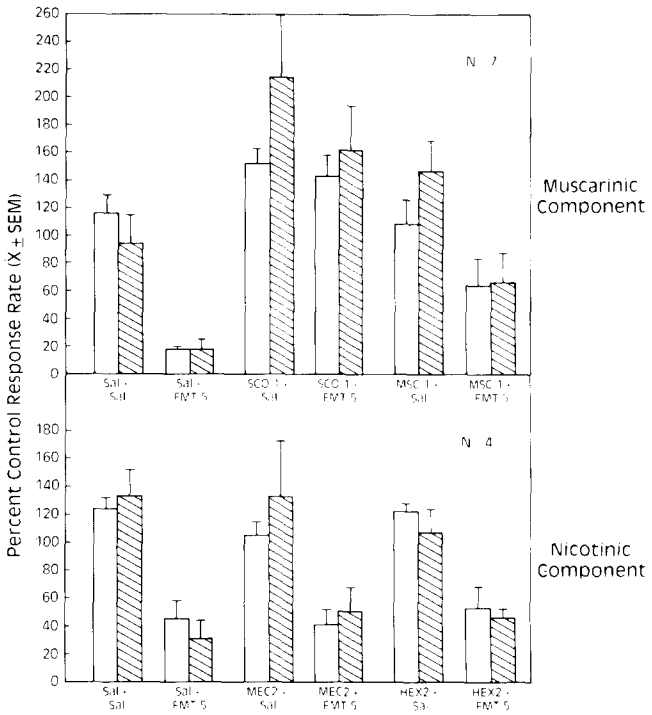

FIG. 2. Effects of combinations of FMT with muscarinic (upper panel) and nicotinic (lower panel) cholinergic blocking agents on response rates maintained under FI 1-min (open bars) and under FI 5-min (hatched bars). For each combination the first compound listed was given 20 min pre-session and the second compound was given 5 min pre-session. Control response rates averaged 0.57 response/sec (FI 1-min) and 0.18 response/sec (FI 5-min) for the muscarinic determinations, and 0.50 response/sec (FI 1-min) and 0.16 response/sec for the nicotinic determinations.

Data Analysis

The total number of responses occurring within each FI were divided by the elapsed time each FI was in effect in order to calculate overall response rates. The effects of each treatment were expressed relative to each rat's baseline (100% control) performance and then averaged across rats. In addition, responses occurring within successive fifths of each FI were tabulated and measures of within-interval response patterning (index of curvature) were calculated daily for each rat. However, since FMT alone had no effect on this measure (see [7]), these data are not presented.

Chemicals

Formetanate HCl was kindly supplied by Nor-Am Agricultural Products, Inc. (Naperville, IL). All other compounds were purchased from Sigma Chemical Co. (St. Louis, MO). All dosages are expressed in terms of the total salts identified above.

RESULTS

Preliminary results indicated that scopolamine alone (0.05–0.5 mg/kg) produced a biphasic effect on responding,

consisting of rate increases then decreases. Slight rate increases were also observed with methylscopolamine, but at larger dosages (0.5–1 mg/kg). Mecamylamine and hexamethonium were largely without effect over the dosage range studied (1–2 mg/kg). Complete data are not presented for purposes of brevity.

Figure 2 shows the combined effects of FMT and the cholinergic receptor blocking agents. Seven rats were tested with the muscarinic agents, but due to evidence of the development of tolerance to FMT only four were tested with the nicotinic agents. As reported previously [7], FMT alone produced equal rate decreases in both FI components of the multiple schedule. This non-differential change in responding was generally observed following all combinations of the cholinergic blockers with either saline or FMT. An exception was the administration of scopolamine and saline, following which responding under FI 5-min was increased more than responding under FI 1-min. Scopolamine (SCO) produced clear increases in rates of responding (one-tailed Mann-Whitney $U=39$, $n_1=n_2=14$, $p<0.01$), and completely reversed the response rate decreases produced by FMT ($U=0$, $n_1=n_2=14$, $p<0.001$, one-tailed). Methylscopolamine (MSC) also attenuated the effects of FMT ($U=34$, $n_1=n_2=14$, $p<0.01$, one-tailed), although not to the same as did scopolamine ($U=27$, $n_1=n_2=14$, $p<0.001$, one-tailed). In contrast, neither mecamylamine (MEC) nor hexamethonium (HEX) had any influence on the rate-decreasing effects of FMT (for both blockers, $U=19$, $n_1=n_2=8$, $p=0.097$, one-tailed).

DISCUSSION

In the present experiment, FMT produced decreases in response rates that were proportionately equivalent under both FI components of the multiple schedule. These equivalent decreases were obtained despite the fact that baseline response rates in the two components differed appreciably (by a factor of 3). This type of effect has been obtained under other schedule conditions and in a variety of animals following exposure to cholinesterase-inhibiting carbamate and organophosphate compounds (for review, see [5]). Moreover, although FMT produced substantial decreases in overall response rates, the within-interval temporal pattern of responding was not appreciably affected. Similar effects have also been obtained with a wide range of cholinesterase-inhibiting compounds (e.g., [1, 3, 13]). Effects of FMT differ markedly from those obtained with two other formamidines, chlordimeform and amitraz [7].

The results of the present experiment also showed that the response-rate decreasing effects of FMT could be blocked by prior treatment with scopolamine and methylscopolamine but not with mecamylamine or hexamethonium. Evidence that muscarinic receptors are primarily involved in the behavioral disruption produced by other carbamates, such as physostigmine, has been reported previously (e.g., [8,11]). Our data further indicate that peripheral actions play an important role in the behavioral effects of FMT, as has also been observed for physostigmine [11,13]. Although it is of course possible that larger dosages of the nicotinic blockers may have attenuated the response-rate decrease produced by FMT, the dosage of mecamylamine used in this experiment was twice as great as that shown by Stitzer *et al.* [9] in rats to completely block the suppression of FI-reinforced responding produced by nicotine. Our results, therefore, suggest that FMT acts principally as an indirect muscarinic agonist in altering behavior.

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