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Effects of Caffeine and Chlor-desmethyldiazepam on Fighting Behavior of Mice With Different Reactivity Baselines

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TRAVERSA, U., L. DE ANGELIS, R. DELLA LOGGIA, M. BERTOLISSI, G. NARDINI AND R. VERTUA. *Effects of caffeine and chlor-desmethyldiazepam on fighting behavior of mice with different reactivity baselines.* PHARMACOL BIOCHEM BEHAV **23**(2) 237–241, 1985.—The effects of various doses of caffeine and of chlor-desmethyldiazepam on footshock-induced aggressive behavior were examined in mice with different baselines of aggressiveness. Caffeine significantly increased the number of fighting episodes with all the doses tested. This was more evident in mice with low rather than in those with high basal rates of agonistic response. Caffeine caused the appearance of minimal convulsive signs in mice subjected to a threshold electroshock which did not produce any seizure in the controls; it also increased metrazol toxicity. Chlor-desmethyldiazepam enhanced fighting behavior at doses of 0.04 and 0.08 mg/kg, but decreased it at 1.25 mg/kg. The first two doses produced the same effects as caffeine on electroshock test, but did not influence metrazol toxicity.

Caffeine Benzodiazepine Fighting behavior

SEVERAL drugs are important in relation to aggressive behavior [21]. Among them, caffeine and benzodiazepines are of particular interest. Caffeine has been little studied to date in correlation with aggressiveness. This is quite surprising because caffeine is one of the most commonly used drugs, although it is often regarded as little more than a dietary constituent rather than as a drug. In contrast, a vast literature is available on the effect of amphetamines on aggressiveness [18,21].

Benzodiazepines are very widely used drugs and several reports indicate a biphasic effect on aggression: low doses enhance, higher doses decrease it. However, the majority of studies are focused on the antiaggressive effects of these drugs and therefore very aggressive animals and high dose levels have been employed [21].

It is also believed that drug effects may be determined primarily by the baseline rate of behavior emitted by organisms, with different rate-dependency functions for different classes of drugs [17]. The aim of the present research was to investigate the effects of caffeine and chlordesmethyldiazepam on fighting behavior in mice with different baselines of response. Chlor-desmethyldiazepam has been chosen as a representative drug in the anxiolytic category, since it has been thoroughly studied in this laboratory [6, 7, 27].

METHOD

Animals

Male Albino Swiss-NOS mice, weighing about 24–27 g, were used. They were housed in groups of sixteen, with food and water available ad lib. The cages were located in the experiment room 24 hr before the tests.

Drugs

The following drugs were used: caffeine, d-amphetamine (Merck, Darmstadt), chlor-desmethyldiazepam or 7-chlor-5-(2′-chlorphenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one (Ravizza, Muggió), and metrazol (Knoll, Liestal).

Caffeine and d-amphetamine, as bases, were administered dissolved in distilled water. Chlor-desmethyldiazepam (Cl-DMDZ) was dissolved in a solvent made up of 30 ml propylene glycol, 24 ml ethanol 96%, 0.2 g KH_2PO_4, 0.1 g $Na_2HPO_4 \cdot 12H_2O$ and distilled water ad 100 ml.

The different amounts of the benzodiazepine were dissolved in a fixed volume of solvent, 0.25 ml/kg, since this quantity was shown experimentally not to interfere with the tests (unpublished data). All the drugs were injected intraperitoneally, 60 min before the experiments.

The animals were randomly assigned to different drug

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TABLE 1

EFFECTS OF CAFFEINE AND d-AMPHETAMINE ON SHOCK-INDUCED AGGRESSIVE BEHAVIOR: POSITIVE MICE†

groups	mg/kg IP	pairs mice	fighting episodes during 3-min trial median (quartiles)
Controls		92	13 (8–19)
caffeine	1.00	56	17 (11–23)*
caffeine	2.50	41	19.5 (17–27)*
d-amphetamine	0.63	30	22 (19–25)*

*Significant differences from controls are determined by using Kruskal-Wallis one-way ANOVA followed by distribution-free rank sums multiple comparisons at a one-tail experimentwise error rate of 0.05.

†Positive mice: mice exhibiting at least one fighting episode during 1-min preselection period.

treatments, which were scheduled in different periods. Each single experiment was repeated at least twice. The drugs were coded so that the observers scoring the test had no knowledge of the drug treatment and dose.

Footshock-Induced Fighting Behavior

This behavior was induced in randomly selected pairs of animals by subjecting them to a footshock for a 3-min period [23,26]. The parameters for electrical stimulation consisted of an interrupted direct 90 volt stimulus intensity of 1 msec duration, administered at a frequency of 5 shocks per sec. The pairs of mice were preselected as "positive or responsive" when they exhibited at least one fighting episode within a 1-min period of shock. It must be noted that a fighting episode occurred when the mice converged abruptly to close quarters, stood face to face on their hind legs, and sparred and bit savagely at each other.

The strain used did not have a very high level of aggressive behavior, since only 30% of the mice were positive. The other 70%, which showed no boxing postures during the pre-testing, were considered "negative or non-responsive." The footshock was applied to both positive and negative pairs of mice, two days after the preselection.

The following parameters were scored: number of fighting episodes and percentage of negative mice developing fighting behavior.

Low Frequency Electroshock Seizure

This experiment was performed by using an ECT Unit (Basile, Comerio, Varese) and stimulation, delivered through auricular electrodes, with the following parameters: frequency 50 Hz, pulse width 0.8 msec, shock duration 0.2 sec, and a current intensity of 8 mA. These stimulation characteristics were determined experimentally as the maximum amount of current not producing any signs of minimal seizure. The minimal signs of seizure were similar to those of low-frequency electroshock seizure threshold described by Swinyard [25], and were characterized by the following manifestations: the posture was awkward but upright, the forelimbs were crossed or flexed, the hindlimbs spread apart, and the tail was vertical.

TABLE 2

EFFECTS OF CAFFEINE, d-AMPHETAMINE AND CHLOR-DESMETHYLDIAZEPAM ON SHOCK-INDUCED AGGRESSIVE BEHAVIOR: NEGATIVE MICE†

groups	mg/kg IP	% responsive pairs‡	fighting episodes of responsive pairs during 3-min trial median (quartiles)
Controls		56 (16)§	3 (2–4.5)
Caffeine	2.50	71 (48)	12 (8–17.5)*
	10.00	81 (16)	9.5 (6–20)*
	20.00	69 (16)	10 (6–14)*
Controls		56 (16)	2.5 (1.5–3.5)
d-Amphetamine	0.33	69 (16)	10 (3.5–14)*
	0.63	69 (32)	9 (5.5–16.5)*
	1.25	88 (16)	12 (10–13)*
	2.50	81 (16)	13 (9.5–16.5)*
Controls		56 (16)	2 (1–2.5)
Chlor-desmethyl-diazepam	0.005	56 (16)	2 (1.5–3.5)
	0.04	71 (24)	5 (2–9)*
	0.08	50 (40)	4 (2–11)*
	0.16	44 (16)	3.5 (2–5)
	1.25	13 (16)¶	1 (0.5–2)

*Significant differences from the respective controls as determined by using Kruskall-Wallis ANOVAs followed by distribution-free multiple comparisons of rank sums at a one-tail experimentwise error rate of 0.05.

†Negative animals: mice showing no fighting episode during 1-min preselection period.

‡Responsive pairs: mice developing at least 1 fighting episode during 3-min trial period.

§Number of pairs tested is shown in parentheses.

¶Significant difference from the respective controls as determined by Chi-square test at an experimentwise error rate of 0.05.

Metrazol Toxicity

Caffeine (10.0 mg/kg), d-amphetamine (2.5 mg/kg), and Cl-DMDZ (0.04, 0.08 mg/kg) were administered IP 60 min before the convulsant, and the influence on the metrazol LD50's was evaluated [20]. The metrazol was administered intraperitoneally. Twenty series of 8 mice each were used, and 4 dose levels for each LD50 value were employed.

Statistical Analysis

Frequencies of responsive pairs were compared by using a Chi-square test. Numbers of fighting episodes of responsive pairs were compared using a Kruskal-Wallis one-way ANOVA followed by distribution-free multiple comparisons of rank sums for K groups as indicated by Hollander and Wolfe [14]. In both cases a one-tail experimentwise error rate of 0.05 was used (0.05/k−1). Frequencies of convulsive responses to electroshock were compared by using Fischer's exact probability test.

RESULTS

In Table 1 the effects of caffeine and d-amphetamine on the fighting behavior of positive mice are reported. Caffeine

TABLE 3

EFFECTS OF CAFFEINE AND CHLOR-DESMETHYLDIAZEPAM ON RESPONSE TO LOW FREQUENCY ELECTROSHOCK IN MICE

groups	mg/kg IP	responsive animals†
Controls		0/20
Caffeine	2.50	6/10*
	5.00	7/10*
	10.00	8/10*
	20.00	6/10*
Chlor-desmethyldiazepam	0.04	7/10*
	0.08	7/10*
	1.25	0/10

*Significant ($p<0.0004$) difference from controls (Fisher's exact probability test).

†Responsive animals: mice showing at least one convulsive phenomenon (see Method section).

TABLE 4

EFFECTS OF CAFFEINE, d-AMPHETAMINE AND CHLOR-DESMETHYLDIAZEPAM ON METRAZOL LETHAL DOSE 50% IN MICE

groups	mg/kg IP	metrazol mg/kg IP LD50 and confidence limits (95%)
Controls		81.0 (88.3–74.3)
Caffeine	10.00	62.0 (66.9–57.4)
d-Amphetamine	2.50	58.0 (63.8–52.4)
Chlor-desmethyl-diazepam	0.04	81.0 (88.3–74.3)
	0.08	80.0 (86.4–74.0)

LD50 and confidence limits (95%) calculated according to Litchfield and Wilcoxon [20].

induced a significant dose-dependent increase in the number of fighting episodes. It appeared, also, that caffeine was about 4 times less potent than d-amphetamine, the reference drug, since a similar number of fighting episodes (median 19.5 and 22) was obtained at the doses of 2.5 mg/kg of caffeine and 0.63 mg/kg of d-amphetamine.

In Table 2 the effects of different doses of caffeine, d-amphetamine and chlor-desmethyldiazepam, on the shock-induced fighting behavior of negative mice are reported. As previously stated, negative mice were basically non-responsive, since they did not react during 1-min of preselection. Actually these animals, when subjected to a 3-min period under the same conditions as in the preselective shock, showed a complete lack of fighting episodes in 44% of the cases, undergoing the shock passively. Fifty-six percent of the animals showed a median number of fighting episodes of 2.5 (quartiles 1.5–4.5), a value close to that reported in the literature as a non-aggressiveness index [26]. Caffeine, at doses ranging from 2.5 to 20.0 mg/kg, did not raise the percentage of pairs of mice shifting from non-responsive to responsive significantly, but did increase the number of fighting episodes significantly, reaching values close to the median of positive controls. A similar pattern of effects was obtained with amphetamine. The doses of 0.04 and 0.08 mg/kg of chlor-desmethyldiazepam significantly increased the average number of fighting episodes, while the doses of 0.005 and 0.16 mg/kg failed to influence this parameter. The dose of 1.25 mg/kg significantly decreased the percentage of pairs of mice shifting from non-responsive to responsive in comparison with the controls. In fact only 2 out of 16 pairs displayed 1 and 3 fighting episodes respectively, and 87% of them remained at zero-baseline.

In Table 3 the effects of caffeine and chlordesmethyldiazepam on low frequency electroshock seizure is shown. Caffeine, at any of four dosages tested (2.5–20.0 mg/kg), as well as Cl-DMDZ at 0.04 and 0.08 mg/kg, caused the appearance of minimal convulsive signs in 60–80% of the animals. The dose of 1.25 mg/kg Cl-DMDZ did not produce any convulsive signs since this concentration completely antagonized convulsions induced by metrazol [27].

As indicated in Table 4 caffeine had a significant effect on metrazol LD50's: caffeine increased metrazol toxicity. Also in this test caffeine proved to be approximately 4 times less potent than d-amphetamine. Chlor-desmethyldiazepam did not affect metrazol LD50's at the doses of 0.04 and 0.08 mg/kg.

DISCUSSION

Caffeine clearly increased footshock-induced fighting behavior, both in positive and in negative mice. The effect of caffeine was greater in mice showing low baseline levels of fighting (negative mice). In fact, caffeine increased fighting by a factor of 3–4 in these mice and by a factor of 1.5 in mice with high baseline of fighting (positive mice). However, the lack of significant difference between doses suggested an all-or-none effect. A similar trend of activity was displayed by d-amphetamine.

The increase of aggressive behavior was thus more evident in animals showing a natural low reactivity. This agrees with the observations of some authors who stated that the stimulation of aggressive behavior also depends on its initial level which should be rather low [13,21]. Such results may be somewhat related to the rate-dependency phenomenon [24], at least as far as its analogies to the law of initial values are concerned [31]. The rate-dependency principle implicitly assumes that there will be individual factors that contribute to drug effects. In our case the intensity of the effect of a given dose of caffeine appeared to be determined mainly by an intrinsic animal factor (baseline reactivity) which in turn affects the rate of control response.

Reports in the literature show that in aggressive mice low doses of benzodiazepines are required to stimulate fighting also after a single, acute treatment [4, 12, 19, 22]. In our experimental design, chlor-desmethyldiazepam was tested employing non-aggressive mice, since different control rates of responding may change quantitatively the behavioral effect of a drug [9]. Low doses (0.04 and 0.08 mg/kg) of Cl-DMDZ produced a significant increase in the number of fighting episodes in mice with low levels of response, thus exhibiting the same type of effect described in the literature in mice with higher baseline, treated with low doses of benzodiazepines.

It is interesting to note that the doses of Cl-DMDZ, which in previous experiments with the hole-board test [7] were

defined as inhibiting (1.25 mg/kg), anxiolytic (0.04 and 0.08 mg/kg) or indifferent (0.005 and 0.16 mg/kg), displayed a parallel effect on footshock-induced behavior. The dose of 1.25 mg/kg lacked any significant effect on mice already having a zero-baseline of fighting, but revealed its depressive effect by significantly lowering the percentage of pairs of mice shifting from non-responsive to responsive in comparison with the controls. On the other hand, this dose inhibited fighting behavior in positive mice [7]. The anxiolytic doses, which caused an increase in explored holes by decreasing emotionality, also increased the number of fighting episodes in non-aggressive mice. This behavioral enhnacement in both experimental situations seems to reflect a disinhibitory action exerted by low doses of the drug.

The observed parallelism between the effects of different doses of Cl-DMDZ in these different experimental situations was evident in the effects of caffeine too. In fact caffeine increases ambulation and exploration in open-field [8,15] at dose levels comparable with those which raised the fighting in animals subjected to footshock.

Moreover, caffeine and Cl-DMDZ caused the appearance of minimal convulsive signs at the same range of doses which increased aggressiveness in negative mice.

The present findings, compared with those described in the literature and already cited [7, 8, 15] indicate that, whatever experimental schedule is used, the facilitating effects of low doses of benzodiazepines and/or stimulating effects of caffeine could enhance performance by an elevation of reactive state of animals. This presumed change of the activation state might be partially mediated by changes of those neurochemicals which interact with, and constrain, behavior.

So, the same variations in turnover of neurotransmitters influencing and controlling different forms of aggression [10, 16, 28], can also affect other behaviors. For instance, neurochemical studies suggest that caffeine may promote synthesis and/or release of catecholamines [29], and inhibition of serotoninergic neurotransmission [2,11]. These neurochemical variations are related to an increase in locomotor activity [30] and are also involved in the control of aggressiveness [16]. Therefore, they may also be important for the stimulation of caffeine-induced agonistic behavior.

The data discussed so far can be interpreted considering an additional hypothesis. Caffeine is an antagonist for A1 subtype of adenosine receptor, whose activation by specific agonists behaviorally produces a depression of locomotor activity [3]. Thus caffeine may stimulate by virtue of its ability to antagonize adenosine [5], which in its turn has an inhibitory neurophysiological role in vivo [1]. The affinities of methylxanthines in competing for A1 adenosine receptor parallel their potencies as locomotor stimulants [3]. Therefore, it is possible that caffeine may regulate the level of activation in animals also by means of its antagonistic action on adenosine.

Although a considerable body of evidence exists supporting an interaction between benzodiazepines and adenosine system [32], it is questionable whether the facilitating effects of anxiolytic low doses of chlordesmethyldiazepam result from this interaction. In fact, findings obtained by molecular studies of reciprocal interaction between benzodiazepines and A1-adenosine agonists on the specific receptor systems are not convincing [33]. Indeed, behavioral studies show that the combination of a low stimulant dose of diazepam and a low stimulant dose of phenylisopropyladenosine (A1-agonist) give a locomotor depression much as a larger dose of diazepam or a larger dose of phenylisopropyladenosine would produce [3]. Moreover, a combination of a low stimulant dose of chlor-desmethyldiazepam and a stimulant dose of caffeine produce an enhancement of the exploration in hole-board test lower than that obtained by the administration of the two drugs, singularly considered [8].

These findings could suggest some connections in the action of these drugs, but do not support the idea that facilitating anxiolytic effects of benzodiazepines may be mediated by an adenosine system.

Therefore from these observations it could be hypothesized that the effects of caffeine and chlordesmethyldiazepam reported in the present study might be mediated by different mechanisms of action even though the two drugs have similar effects on certain aspects of animal behavior.

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