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Drug Discrimination in Rats: Effects of Mixtures of Ditran and Cholinesterase Inhibitors

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JÄRBE, T. U. C. AND J. O. JOHANSSON. *Drug discrimination in rats: effects of mixtures of ditran and cholinesterase inhibitors.* PHARMAC. BIOCHEM. BEHAV. 4(2) 151–157, 1976. – Groups of rats were trained in a T-shaped maze to discriminate the effects produced by IP injections of ditran (1.60 mg/kg), either when given singly, or when combined with the acetylcholinesterase inhibitors neostigmine (0.25 mg/kg) or physostigmine (0.50 and 1.00 mg/kg), from the nondrug condition (saline). The results from this state-dependency (StD) model indicated that acquisition of the drug discrimination was similar for the 4 groups of rats. After drug discrimination was established the rats were tested with various drug combinations. Physostigmine (0.50 and 1.00 mg/kg) challenge reversed drug discrimination among rats trained with ditran solely or the ditran plus neostigmine combination. There was no antagonism among the ditran plus physostigmine trained rats. Involvement of the C.N.S. is implicated since tests with neostigmine did not upset ditran discrimination. In addition, survival rate of physostigmine treated mice is increased with ditran. In conclusion, this study indicates the usefulness of employing both training and transfer test procedures when evaluating antagonism in this StD model.

State-dependency Ditran Physostigmine Neostigmine Drug interaction

IT is well recognized that animals can learn to make differential responses contingent upon whether the animal is drugged or not. Drug discrimination or state-dependent (StD) behavior has been tested with several classes of compounds. A central mode of action is implicated since peripherally acting drugs do not produce response control rapidly [25, 26, 27].

Several StD studies have demonstrated that when an appropriate antagonist is applied together with the training compound the drug contingent discrimination is temporarily upset i.e. the animals make the vehicle contingent choice at the subsequent test. Using the drug discriminative model we recently performed a series of challenge tests with presumed antagonists against ditran, a drug with marked anticholinergic activity [1,16], and it was found that the tertiary anticholinesterase agent physostigmine, but not the quaternary agent neostigmine [10], reversed the ditran produced behavior in rats [13]. In this case drug discrimination was established before testing began. In the present study, on the other hand, we have examined whether drug discrimination would appear when rats were required to differentiate mixtures of ditran and cholinesterase inhibitors (physostigmine and neostigmine) from a saline condition already from the start of the discriminative training. Such an approach may be useful for determining whether antagonism of drug discrimination results in a state similar to that created by the vehicle condition or produces a third state which in itself has discriminative properties.

METHOD

Animals

Thirty-two adult male albino Sprague-Dawley rats, obtained through a commercial breeder (Anticimex, Sollentuna, Sweden), with an average weight of 427 g at the start of the experiment, were used. The rats were individually housed in acrylic animal cages with free access to tap water and pelleted food (Anticimex: Type 210).

Apparatus

The shape and size of the T-maze used corresponds to a water tank previously described [12]. The animals were trained to escape electric shock (120 VAC) in the maze. Shock was applied to the grid floor of the maze at all points. Only by running to the goal alley could the rat escape the shock by jumping off the grid into an acrylic box which had been placed adjacent to the maze exit. The closest distance to be traversed to escape shocks was 90 cm. A 60 W light bulb was placed over the choice area, the 15 x 15 cm junction of the three alleys.

Procedure

The aim with the discriminative training was to see if the rats could learn to respond differentially i.e. turning left or right in the T-maze, on the sole basis of being drugged (D) or not drugged (N).

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On each trial an animal was dropped on the activated grid floor of the center alley and could run freely in the maze until it reached the goal alley and jumped off the grid. Exit through the alley designated incorrect was prevented by a barrier not visible to the animal when making the choice (left or right turn). A choice was recorded when the rat had left the choice area with its whole body, excluding the tail.

Each rat received 5 training trials per training session, spaced 30–60 sec apart. On a given session the exit was found on the same side of the maze at all trials. Rats received 1 session per day, 5 days a week. The two states (D and N) alternated according to the DNND, NDDN principle as did the required choice. Thus the correct side of the maze was contingent upon the prevailing state. To minimize the influence of possible position preferences the left side was correct for half the drugged and half the undrugged rats in a group. The opposite side was appropriate for the remaining animals.

Such a training design was followed for 28 successive training sessions. Hereafter, the animals were given test trials (maximum twice weekly) with various combinations of the drugs (cf. result section). Test sessions were run in a balanced order. At test days only 1 trial per rat was run and both exits were accessible in order to minimize the occurrence of new learning. The regular training continued in between the test days.

We used 4 groups of rats of which 1 group was required to discriminate the effects produced by an injection of ditran from the no drug state (saline) whereas the 3 other groups had to discriminate mixtures of ditran plus one of the anticholinesterase inhibitors, either neostigmine or physostigmine, from the no drug state. Further details are given in Table 1, (cf., result section). The number of rats per group was eight. One rat in each of Groups 1, 2, and 3 did not reach the criterion of performing 8 correct first-trial choices out of 10 consecutive training sessions during the initial 28 training sessions and therefore the number of animals used for the test trials in these 3 groups were 21 (n = 7), except at 3 tests in group 3 where only 5 rats were used (2 rats were excluded because of faulty injections).

The ditran and physostigmine doses were the same as those used in a previous study [13]. Other data [2] suggest that neostigmine is somewhat less than twice as potent as physostigmine. Preliminary studies indicated that naive rats would not traverse the maze if 0.50 mg/kg of neostigmine was used unless the shock level was raised. Therefore, we used 0.25 mg/kg.

In a separate experiment, using male mice (descendents of the NMRI strain), dose-effect lines were obtained for physostigmine when given singly and when combined with ditran.

Drugs. Ditran (a mixture of two isomeric compounds: the N-ethyl piperidine and the N-ethylmethylpyrollidine derivatives, Lakeside Lab.), neostigmine methylsulfate (AB Leo), and physostigmine sulfate (Sigma Co.), were dissolved in isotonic saline. Doses were calculated as salts and only freshly prepared drug mixtures were used. The injections were given intraperitoneally (IP) in a constant volume of 0.8 ml per animal 15 min prior to the sessions. The non-drugged condition consisted of an injection of the corresponding volume of the vehicle. In mice the drugs were also given IP but in a volume of 10 ml/kg.

Data analysis. Analysis of variance was used to evaluate acquisition data, and chi-square analysis was used for comparisons of interest with respect to test trial data [18,29]. The conceptual unit is error rate per comparison for the chi square test although $\alpha(p \leq 0.01)$ was evenly split between comparisons within the D and N distributions respectively. Mice data were evaluated with the method by Litchfield and Wilcoxin [19].

RESULTS

The formation of the drug discriminative control is portrayed in Fig. 1. From the analysis of variance, the details of which are found in Table 1, we conclude that the rapidity in establishing drug discrimination was similar for all groups, though the Group 4 animals seem somewhat superior in this respect. Thus, the differential treatments (drug vs. no drug) made it possible for the rats to make a correct choice, thereby finding the maze exit, already on the first trial of the daily training sessions. Consequently, the term drug responding indicates that the drug associated arm of the maze was choosen whereas a no drug response or choice means that an animal went to the saline associated arm.

Table 2 shows that rats trained to discriminate between ditran and saline (Group 1) showed a significant preference for the saline arm of the maze when subsequently tested with ditran plus physostigmine. Thus, physostigmine reversed the ditran contingent responding among Group 1 animals.

In Table 3 it is shown that such reversal of ditran responding was also apparent in animals (Group 2: DT + NS) that were experienced with the quaternary anticholinesterase agent, neostigmine. It can also be seen from Table 3 that Group 2 animals still choose the drug associated side of the maze even when tested with twice (0.50 mg/kg) the training dose of neostigmine. Table 3 further suggests that discrimination in Group 2 rats can be accounted for by ditran solely since only one rat choose the drug associated arm when tested separately with neostigmine (0.25 mg/kg) whereas treatment with ditran only (1.60 mg/kg) resulted in a level of drug responding comparable to that seen during the drugged training sessions. Thus, ditran produced drug discriminable effects whereas neostigmine did not.

Table 4 shows that neither of the training doses of ditran (1.60 mg/kg) or physostigmine (0.50 mg/kg), when given separately, resulted in more than 50% drug responses in Group 3 rats (DT + PS 0.5). Additional separate treatments with ditran (3.20 mg/kg), but not with physostigmine (1.00–2.00 mg/kg), yielded a high percentage of responses into the drug associated arm of the maze. Thus, it appears that these animals were more familiar with the ditran rather than the physostigmine cues.

Table 5 shows that neither by increasing the dose of physostigmine up to 2.00 mg/kg and maintaining the training dose of ditran (1.60 mg/kg) or by increasing the dose of ditran up to 3.20 mg/kg and maintaining the training dose of physostigmine (0.50 mg/kg) was there any evidence of a preference for the saline arm among Group 3 rats.

A comparison of the test results noted with the higher doses of physostigmine (1.00 and 2.00 mg/kg), presented in Tables 4 and 5, reveals that when ditran was given together with the anticholinesterase compound there was no decline in drug associated responding whereas rather few drug responses were found when physostigmine was given singly.

In the Group 4 (DT + PS 1.0) animals a separate treat-

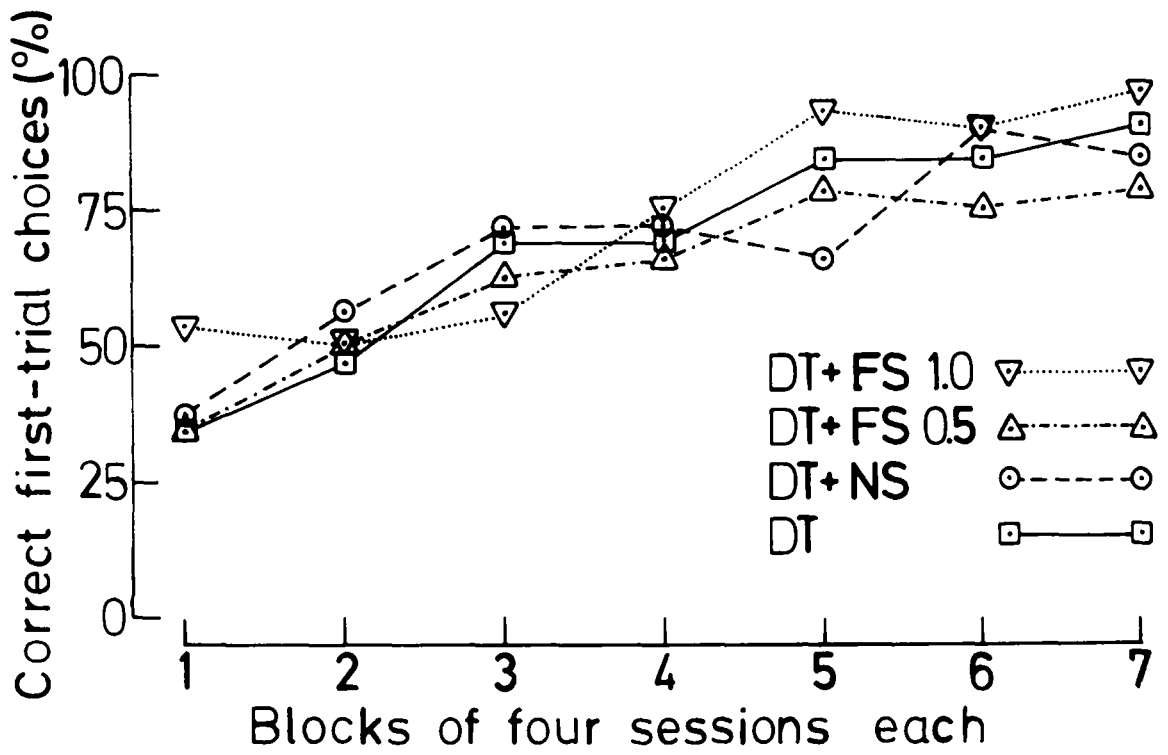

FIG. 1. Formation of drug discrimination. Ordinate, percent correct first-trial choices; abscissa, number of training sessions. Abbreviations are explained in Table 1.

TABLE 1

TRAINING CONDITIONS AND RESULTS FOR THE DRUG DISCRIMINATIVE TRAINING*

Group (n = 8)	1 (DT)	2 (DT + NS)	3 (DT + PS 0.5)	4 (DT + PS 1.0)
State D	Ditran 1.60 –	Ditran 1.60 + Neostigmine 0.25	Ditran 1.60 + Physostigmine 0.50	Ditran 1.60 + Physostigmine 1.00
State N	Saline	Saline	Saline	Saline
NCR 28†	19.25 (3.45)	19.13 (2.36)	17.75 (2.38)	20.63 (2.13)
NSC 8/10‡	19.50 (5.73)	20.00 (5.42)	20.50 (4.78)	19.75 (3.77)

*The rats were trained to discriminate a drugged (D) state from a non-drugged (N) state during 28 sessions. A state is arbitrarily defined as the effects produced by either a drug- or a saline injection given IP in a constant volume of 0.8 ml per animal 15 min prior to the sessions. Doses are expressed as mg/kg body weight.

†Average total number of correct first-trial choices and standard deviations (within brackets) performed by the rats during the 28 training sessions, $F(3,28) = 1.60$, $p>0.05$.

‡Average number of sessions and standard deviations (within brackets) required by the rats to complete a criterion of performing 8 correct first-trial choices out of 10 consecutive training sessions, $F(3,28) = 0.06$, $p>0.05$.

ment (2 × n observations) with the training dose of physostigmine (1.00 mg/kg) maintained drug responding (87.5%) whereas a corresponding treatment with ditran (1.60 mg/kg) did not (25%). The corresponding baseline values were: D state = 96.9% and N state = 96.9% correct first-trial responses (the number of observations were 32 in each case and n = 8).

The results of administrations of physostigmine when given singly and when combined with ditran (1.60 mg/kg) suggest that the survival rate of mice was increased 3 times when ditran was given concomitant with physostigmine. The potency ratio and 95% confidence limits (CL) were estimated to be 3.79 (3.03–4.74), i.e. the ratio between LD_{50} = 3.60 (mg/kg) for physostigmine plus ditran (95%

TABLE 2

CHALLENGE OF DITRAN CONTROLLED BEHAVIOR BY PHYSOSTIGMINE (GROUP 1)

Drug 1	Dose (mg/kg)		Drug 2	Dose (mg/kg)	No. of Trials	% First-Trial Choices Into Correct Arms	
						DT	Saline
N = Saline	–		–	–	21	14.29*	85.71
D = Ditran (DT)	1.6		–	–	21	85.71	14.29†
T = Ditran	1.6	+	Physostigmine	0.5	14	21.43*	78.57
T = Ditran	1.6	+	Physostigmine	1.0	14	21.43*	78.57

*Probability of difference from corresponding ditran (DT) score being due to chance; $p<0.001$, Chi-square test.
†Probability of difference from corresponding saline (N) score being due to chance; $p<0.001$, Chi-square test.
N = no drug state; D = drug state; T = test state

TABLE 3

TRANSFER TESTS WITH DITRAN AND NEOSTIGMINE AND CHALLENGE TESTS WITH NEOSTIGMINE AND PHYSOSTIGMINE IN GROUP 2 (DT + NS)

Drug 1	Dose (mg/kg)		Drug 2	Dose (mg/kg)	No. of Trials	% First-Trial Choices Into Correct Arms	
						DT + NS	Saline
N = Saline	-		–	–	76	3.95*	96.05
D = Ditran (DT)	1.6	+	Neostigmine (NS)	0.25	76	85.53	14.47†
T = Ditran	1.6		–	–	14	100.00	0.00
T = Neostigmine	0.25		–	–	14	7.14*	92.86
T = Ditran	1.6	+	Neostigmine	0.50	14	78.57	21.43†
T = Ditran	1.6	+	Physostigmine	0.50	13	30.77*	71.43†
T = Ditran	1.6	+	Physostigmine	1.00	14	21.43*	78.57

*Probability of difference from corresponding ditran + neostigmine (DT + NS) score being due to chance; $p<0.001$, Chi-square test.
†Probability of difference from corresponding saline (N) score being due to chance; $p<0.001$, Chi-square test.
N = no drug state; D = drug state; T = test state

CL: 3.21–4.03) and LD_{50} = 0.95 (mg/kg) for physostigmine alone (95% CL: 0.79–1.15). Data were not significantly heterogeneous (χ^2 = 4.0 and 1.3 respectively, df = 3; $p>0.05$) and the curves did not deviate significantly from parallelism ($p>0.05$). The animals were observed for at least 12 hr but no animal died after a longer period than 15 min postinjection. There were 10 treatments, 5 with physostigmine alone (dose range: 0.50–1.50 mg/kg) and 5 with physostigmine (dose range: 1.00–5.00 mg/kg) plus ditran (1.60 mg/kg). Eight mice were used for each treatment level. On the basis of these LD_{50} determinations we conclude that ditran, like the Belladonna alkaloids, possesses anticholinergic activity, thereby reducing the lethality of physostigmine.

TABLE 4

TRANSFER TESTS WITH DITRAN AND PHYSOSTIGMINE IN GROUP 3 (DT + PS 0.5)

Drug 1	Dose (mg/kg)		Drug 2	Dose (mg/kg)	No. of Trials	% First-Trial Choices Into Correct Arms	
						DT + PS 0.5	Saline
N = Saline	–		–	–	106	8.26*	91.74
D = Ditran (DT)	1.60	+	Physostigmine (PS)	0.50	108	90.74	9.26‡
T = Ditran	1.60		–	–	28	50.00*	50.00‡
T = Ditran	3.20		–	–	10	80.00	20.00‡
T = Physostigmine	0.50		–	–	14	35.71*	64.29†
T = Physostigmine	1.00		–	–	14	35.71*	64.29†
T = Physostigmine	2.00		–	–	12	25.00*	75.00

*Probability of difference from corresponding ditran + physostigmine (DT + PS 0.5) score being due to chance; $p<0.001$, Chi-square test.

Probability of difference from corresponding saline (N) score being due to chance; †$p<0.01$, ‡$p<0.001$, Chi-square test.

N = no drug state; D = drug state; T = test state

TABLE 5

CHALLENGE TESTS WITH PHYSOSTIGMINE AND DITRAN IN GROUP 3 (DT + PS 0.5)

Drug 1	Dose (mg/kg)		Drug 2	Dose (mg/kg)	No. of Trials	% First-Trial Choices Into Correct Arms	
						DT + PS 0.5	Saline
N = Saline	–		–	–	106	8.26*	91.74
D = Ditran (DT)	1.60	+	Physostigmine (PS)	0.50	108	90.74	9.26†
T = Ditran	1.60	+	Physostigmine	1.00	14	78.57	21.43†
T = Ditran	1.60	+	Physostigmine	2.00	14	92.86	7.14†
T = Ditran	3.20	+	Physostigmine	0.50	10	100.00	0.00†

*Probability of difference from corresponding ditran + physostigmine (DT + PS 0.5) score being due to chance; $p<0.001$, Chi-square test.

†Probability of difference from corresponding saline (N) score being due to chance; $p<0.001$, Chi-square test.

N = no drug state; D = drug state; T = test state

DISCUSSION

This study has demonstrated that established discrimination based on ditran solely or ditran in combination with neostigmine is reversed by physostigmine. Thus the combined treatment mostly resulted in nondrug responding by the rats. This is in agreement with previous results from this laboratory [13] and would also be expected from many studies demonstrating antagonism between anticholinergics and anticholinesterases both in man [9,17] and animals [3, 6, 20, 21]. Likewise, ditran decreased the lethality of our physostigmine treated mice. Yet, those rats that had to discriminate combinations of ditran and

physostigmine from the start of the discriminative training acquired the task at approximately the same rate as did the rats treated with ditran solely. Thus, when given repeatedly, the ditran plus physostigmine combination was clearly discriminable from the no drug conditions.

The reason for the differential effects are obscure. Pharmacologically the two drugs produce opposing effects. Physostigmine elevates levels of brain acetylcholine (ACh) by inhibiting acetylcholinesterase (AChE) whereas ditran presumably decreases uptake of newly synthesized ACh and inhibits synthesis of ACh [16]. Although this provides a pharmacological basis for explaining the antagonism or reversal among ditran trained rats it seems inadequate to explain the acquisition data.

There are observations suggesting that tolerance may develop during repeated exposure to the cholinesterase inhibitors, e.g., a reduced efficacy of physostigmine in alleviating symptoms in manic patients [11] and demonstrations of subsensitivity to ACh at receptor sites after prolonged exposure of that chemical [4, 8, 23]. Our transfer test data indicated that the rats required to discriminate the physostigmine (0.50 mg/kg) – ditran (1.60 mg/kg) combination somehow had become more familiar with the ditran rather than the physostigmine cues. It was found that drug responding was maintained whenever ditran was given in conjugation with physostigmine whereas separate treatments with the AChE agent only resulted in 25–35% drug responses. Although a separate test with the training dose of ditran (1.60 mg/kg) was insufficient we found that twice this dose maintained drug responding. These data may be interpreted as reflecting development of tolerance to phsysostigmine but at the same time it is clear that drug responding is optimized when both drugs are given concurrently. Besides, a separate test with physostigmine (1.00 mg/kg) in the Group 4 animals (DT + PS 1.0, cf. Table 1) resulted in a level of drug responding close to that noted for the drugged training condition. Also, in a subchronic study where physostigmine 0.75 mg/kg was given twice daily for 5 days no significant differences of ACh levels were found between controls and treated rats [30]. Development of tolerance to physostigmine therefore does not seem likely to explain the differential effects between the training and test data. Anyhow, we do not know which drug effects the animals utilized to respond differentially and since the mechanisms underlying discriminative behavior as such is poorly understood [5, 7, 22, 25, 28, 31], it is perhaps best to defer further theoretical sepculations until more data are available.

Physostigmine was not included as a separate drug in the present study because previous work had indicated that this drug exerted only weak discriminative control [24]. In a separate study we nevertheless found that physostigmine (0.50 mg/kg) could be differentiated from the no drug condition by most rats within 20 sessions (submitted for publication). Moreover, ditran partially reversed the physostigmine contingent responding. As judged from the challenge tests there is thus a reciprocal antagonism between physostigmine and ditran in the present StD model. On the other hand certain dose combinations of the two drugs (0.50 and 1.60 mg/kg in this experiment) obviously produces discriminable effects which appear somewhat different from those induced by separate treatments of the drugs. Thus, it may be fruitful to employ both training and transfer test procedures when evaluating antagonism in the drug discriminative model [14, 15, 28].

A rough indication of an involvement of the central nervous system for maintaining the drug discrimination is provided by the test results with neostigmine because the quaternary ammonium structure of this AChE agent restricts its penetration through cell membrances such as the blood-brain barrier [10]. Thus, challenge of ditran discrimination with neostigmine does not significantly upset drug discrimination as demonstrated here and elsewhere [13]. In addition, physostigmine reversed drug discrimination among Group 2 rats (DT + NS) to a degree comparable to that of the ditran trained rats. This happened in spite of the extensive previous drug history with neostigmine in Group 2 animals. In congruence, neostigmine does not maintain drug responding when tested among physostigmine trained rats (to be published).

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