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Effects of Cerebral Depletion of Norepinephrine on Conditioned Avoidance Responding in Sprague-Dawley and Fischer Rats[1]

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MARTIN, G. E. AND R. J. ELGIN, JR. *Effects of cerebral depletion of norepinephrine on conditioned avoidance responding in Sprague-Dawley and Fischer rats.* PHARMACOL BIOCHEM BEHAV **30**(1) 137–142, 1988.—Block of conditioned avoidance responding (CAR) in the rat is a property of all antipsychotic agents. To determine whether cerebral norepinephrine (NE) is crucial for CAR, the effect of depletion of cerebral NE was examined both during acquisition and retention of a CAR task in Sprague-Dawley and Fischer 344 male rats. In examining acquisition of CAR, DSP-4 [N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine] (50 mg/kg, IP) was given to naive rats from each strain. In one control group, desmethylimipramine (DMI, 20 mg/kg, IP −30 min), which inhibits the uptake of DSP-4 and its subsequent neurotoxic effect, was given prior to DSP-4. After DSP-4, all animals were tested for acquisition of CAR in a discrete trial paradigm in which a lever press avoided a 0.7 mA shock. After the experiment, the rats were sacrificed and the cerebral cortex, striatum, and brain stem were removed for NE and dopamine assay using HPLC. In examining retention of CAR, the effect of DSP-4 on the CAR of trained rats was observed. DSP-4 produced an almost total depletion of cortical NE and about 50% reduction of NE in the brain stem in both strains and in both tests. In the first experiment, DSP-4 failed to significantly diminish CAR acquisition in either strain, although there was a trend towards a DSP-4-induced deficit. Interestingly, DSP-4 caused no decrement in CAR in trained rats of either strain, but did significantly impair further acquisition of CAR in Sprague-Dawley rats. The data demonstrate cerebral NE is not critical for retention of CAR, but suggest a possible role for NE in the acquisition of CAR.

Norepinephrine Conditioned avoidance responding DSP-4

ALL known antipsychotic drugs block the performance of conditioned avoidance responding (CAR) in rats [4, 5, 7, 8,] with a potency directly proportional to the daily clinical recommended dose in man [10]. Since all known antipsychotic drugs displace ligands for the dopamine D-2 binding site with affinities directly proportional to clinically effective dose levels [18], it has generally been inferred that block of CAR is due to block of central dopamine receptors. On the other hand, no antipsychotic agent acts exclusively at dopamine receptors. In fact, many also interact with serotonergic, adrenergic and cholinergic binding sites [11, 12, 16]. It is possible that one or more of these other transmitter systems may play a role both in mediating acquisition or performance of CAR and, as a corollary, in the elaboration of their therapeutic action. It may not be correct to assume that novel agents which block CAR must be antidopaminergic.

A closer examination of this animal model might reveal some new clues as to the mechanism of action of antipsychotic drugs in man. In the present study, the possible role of cerebral norepinephrine (NE) in the mediation of CAR performance in the rat was examined. The effects of DSP-4 [N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine], which selectively and irreversibly depletes cerebral stores of NE following intraperitoneal administration [17], on a discrete trial lever press CAR task was ascertained in two strains of rat. Previously, the effect of DSP-4 on CAR has been studied in both one- and two-way acquisition paradigms in the Sprague-Dawley rat with indications of an impairment of acquisition [1, 2, 15] and of no effect [3]. In the present experiments, DSP-4's actions in the rat were measured during both the acquisition period for the CAR and after animals had been trained in the CAR task using both Sprague-Dawley and Fischer 344 rats. The latter strain was included since it has been reported to perform better in CAR tasks than other strains of rat [5].

METHOD

Experiment 1: Effect of DSP-4 on Acquisition of CAR

Male rats of the Fischer 344 or Sprague-Dawley strains, weighing 230–260 g and purchased from Charles River

[1]A preliminary report of these data was given at the Annual Meeting of the Society of Neuroscience, Dallas, 1985.

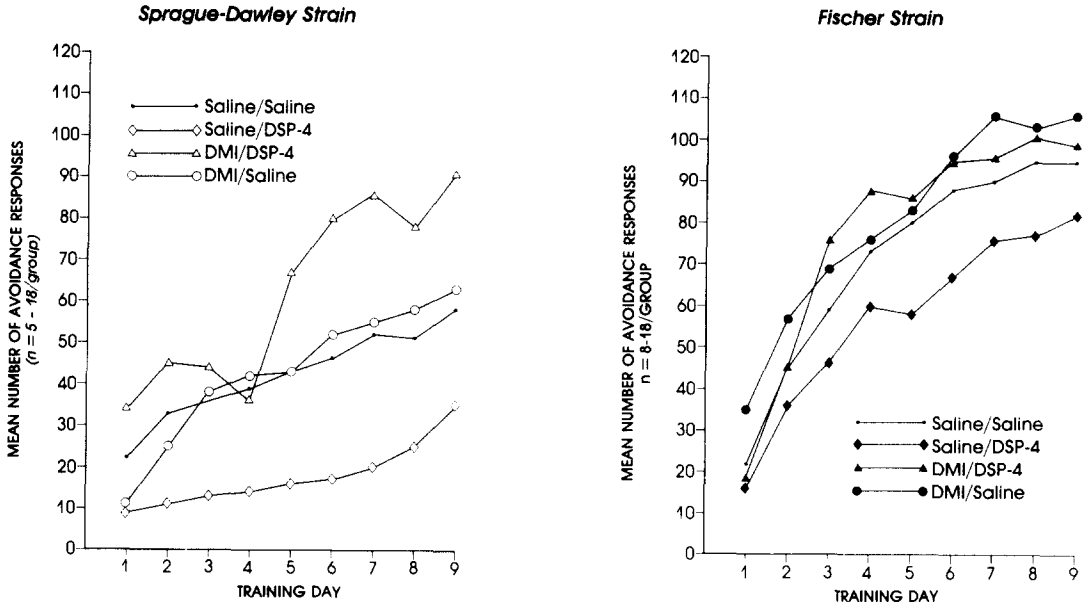

FIG. 1. The mean number of CARs recorded for Fischer 344 and Sprague-Dawley rats on Days 1 through 9 of training. As pretreatment/treatment the groups Fischer/Sprague-Dawley were given: ●—● saline/saline (n=18)/(n=18); ▲—▲/△—△ DMI (20 mg/kg, IP)/DSP-4 (50 mg/kg, IP) (n=9)/(n=7); ◆—◆/◇—◇ saline/DSP-4 (n=8)/(n=5); and ●—●/○—○ DMI/saline (n=10)/(n=10). Standard errors did not exceed 11% of the mean for Fischer rats in any measurement and ranged from 13 to 16% for Sprague-Dawley animals. They were omitted for clarity. Data from each day's testing were compared with the saline/saline control group using Dunnett's multiple comparison test ($p<0.05$) and no significant difference between DSP-4 and saline-treated animals was found.

(Kingston, NY), were used. Each was housed individually with access to food and water ad lib throughout the experiment.

All drugs were administered ten days prior to the start of acquisition testing, since peripheral NE recovers to near control levels at this time after the administration of DSP-4 whereas cerebral NE remains depleted [9]. There were four treatment groups: (1) saline followed 30 min later by saline; (2) saline followed 30 min later by DSP-4 (50 mg/kg, IP); (3) desmethylimipramine (DMI, 20 mg/kg, IP) followed 30 min later by DSP-4 (50 mg/kg, IP); and, (4) DMI (20 mg/kg, IP) followed 30 min later by saline. DMI prevents the uptake of DSP-4 into NE containing cells and prevents the depletion of NE [17]. This group was added to control for effects of DSP-4 that might not be related to its NE depleting action. Each group was composed of 10 rats except for the saline/saline group which consisted of 18 animals. Six of these which performed the poorest in making CARs were sacrificed at the end of this experiment. The remaining animals, which were those animals that had performed the best, were utilized in Experiment II. Results for this experiment are sometimes reported for less than ten animals per group for two reasons. First, DSP-4 (50 mg/kg) was fatal to some animals. Secondly, if the DSP-4 was found to have failed to produce a significant reduction of NE from the control level, the data from that animal were eliminated from the statistical analyses. Identical experiments were carried out using Fischer 344 and Sprague-Dawley rats.

Each rat was trained to perform the CAR task which consisted of a lever press to avoid the 0.7 mA foot shock. A Campden Instrument Ltd. rodent test cage, measuring 22.5×23.4×20 cm (D×W×H), constructed from Plexiglas and stainless steel and housed in a Campden Instruments Ltd. sound-attentuating chamber, was used. A stainless steel lever, positioned 5.94 cm above the grid floor, extended into the cage 1.875 cm. The scrambled shock was delivered via the stainless steel grid floor by a Coulbourn Instruments solid state shocker. The test session consisted of 120 trials spaced evenly over a one-hour period. The conditioning stimuli (paired light and tone) were presented for 15 seconds followed by 10 seconds of shock in the absence of a lever press. Each animal was tested once daily for nine consecutive days. Avoidance responses were compared between groups using analysis of variance with Dunnett's multiple comparison test ($p<0.05$).

At the end of acquisition testing, the rats were sacrificed by cervical dislocation and their brains were removed. The cerebral cortex, corpus striatum and brain stem (pons and medulla) were rapidly dissected on an ice-cold plate and rapidly homogenized in 2% perchloric acid. Following centrifugation, a sample of the supernatant was assayed for NE and dopamine levels using an HPLC system with electrochemical detection. A Biophase ODS reverse-phase column No. 5588 was used. A mobile phase consisting of 20% methanol containing sodium acetate (2.04 g/l), citric acid (7.69 g/l), sodium octyl sulfate (0.15 g/l) and EDTA (0.05 g/l) was used. The samples were read with the electrode set to a potential of 0.65 V using a BAS LC-4B amperometric detector (West Lafayette, IN 47907). The levels of monoamines were compared among groups using an analysis of variance in conjunction with Duncan's multiple range test ($p<0.05$).

Experiment II. Effect of DSP-4 on CAR Performance in Animals Trained in CAR

Animals and training. The best performing male rats from

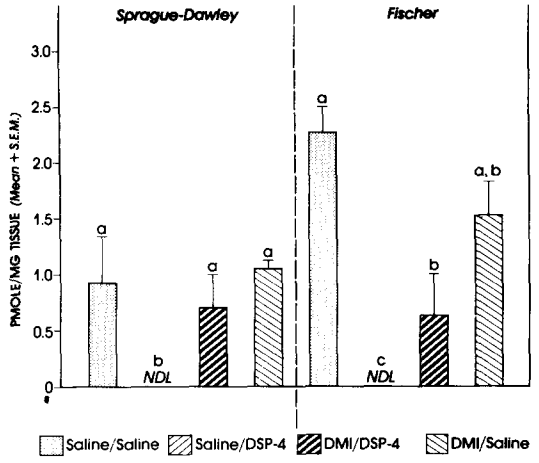

FIG. 2. Mean (+S.E.M.) levels of norepinephrine found in cortical tissue of rats pretreated with saline/saline, saline/DSP-4, DMI/DSP-4, and DMI/saline. Data are shown for Sprague-Dawley (left) and Fischer 344 (right) rats. Statistical comparisons were made within strains and bars that do not share the same letter are statistically different ($p<0.05$, Duncan's multiple range test). NDL=no detectable levels of NE were found in the tissue sample.

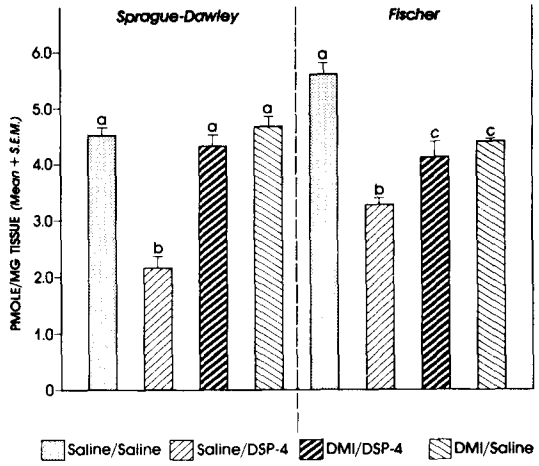

FIG. 3. Mean (+S.E.M.) norepinephrine levels found in tissue from brain stem of Sprague-Dawley (left) and Fischer 344 (right) rats (n=5–8 per treatment). The pretreatment/treatments are indicated as in Fig. 2. Statistical comparisons were drawn within each strain and bars with the same letter do not differ significantly ($p<0.05$, Duncan's multiple range statistic).

the Sprague-Dawley (n=11) or Fischer 344 (n=10) strains were selected from the control group of the previous study. Each group had been given nine one-hour training sessions. About half of the animals were given DSP-4 (n=4 for Fischer 344 rats and n=5 for Sprague-Dawley rats) and the remaining animals were given saline. The groups were matched for CAR performance by rank ordering the rats according to CARs and assigning even numbered animals to one group and odd numbers to the other. Ten days after treatment, four daily CAR sessions were run. The mean number of CARs between DSP-4- and saline-treated groups were compared daily using Student's *t*-test. The cerebral levels of NE and dopamine were measured at the end of the experiment as in the previous experiment.

RESULTS

Experiment I. Effect of DSP-4 on Acquisition of CAR

The mean number of CARs for the four treatment groups are shown for Fischer 344 rats and Sprague-Dawley rats in Fig. 1. Although the acquisition curve for Fischer 344 rats treated with DSP-4 resides below that of the control group for each training session, statistical analyses of the mean number of CARs per group failed to reveal a statistically significant difference between the DSP-4-treated and the control group at any timepoint ($p<0.05$, Dunnett's multiple comparison test). Similarly, Sprague-Dawley rats given DSP-4 seemed to learn the avoidance task at a lower rate than the saline-treated animals (Fig. 1). However, statistical analyses of the results of each training session once again failed to reveal significant differences from the control group for the DSP-4-treated rats. In contrast to the DSP-4-treated animals, the performance of DMI/DSP-4-treated rats was closer to that of control (Fig. 1). In fact, DMI/DSP-4-treated animals of the Sprague-Dawley strain demonstrated a higher than control avoidance response on Days 5 to 9 after treatment, although this difference was not statistically significant.

In contrasting the performance of the two strains of rat in the acquisition testing, the Fischer 344 strain performed

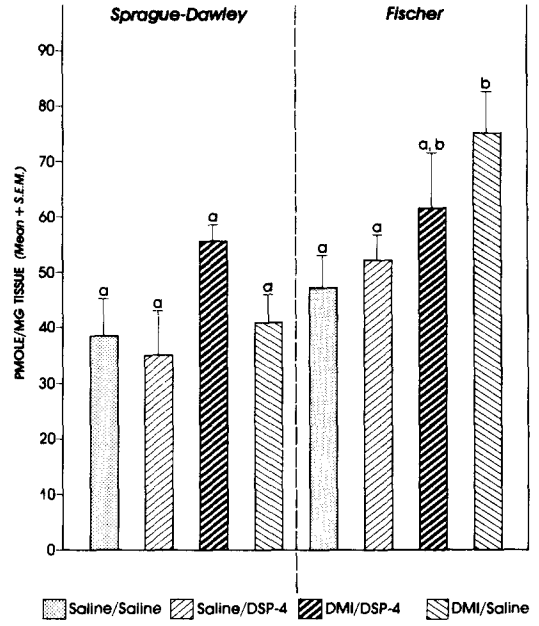

FIG. 4. Mean (+S.E.M.) dopamine levels in the striatum of Sprague-Dawley (left) and Fischer 344 (right) rats (n=5–8 per treatment). Pretreatment/treatment regimens are the same as Fig. 3. Statistical comparisons are drawn within strains. Bars with the same letter above them do not differ significantly ($p<0.05$, Duncan's multiple range statistic).

statistically better than the Sprague-Dawley animals when the groups treated with saline/saline were compared. The Fischer 344 animals were registering 95.4±6.1 (mean± S.E.M.) CARs to 58.1±8.7 for the Sprague-Dawley strain after nine days of testing (Fig. 1).

DSP-4 depleted cortical NE below the levels of detection in both strains of rat as shown in Fig. 2. In the Sprague-Dawley rats, DMI prevented the cortical depletion of NE completely. In the Fischer 344 animals given DMI, on the other hand, there

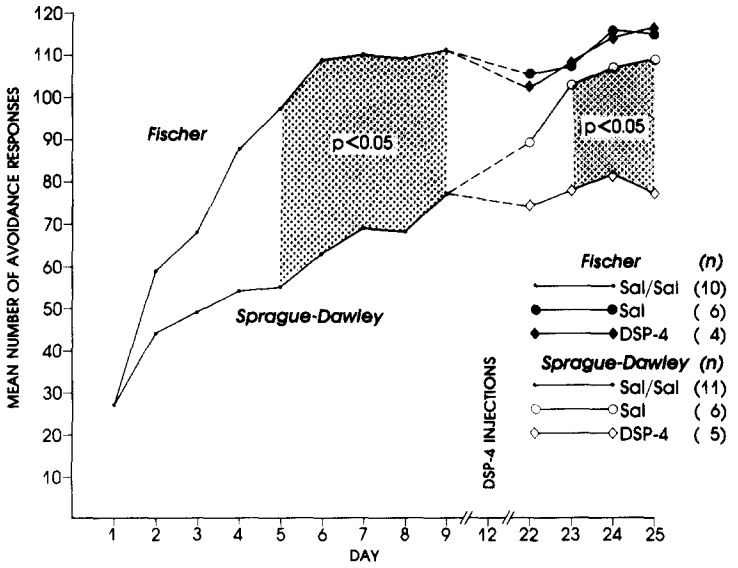

FIG. 5. Mean number of CARs performed by Sprague-Dawley and Fischer 344 rats before and after administration of DSP-4 (50 mg/kg, IP). Stippled areas indicate: (1) significant differences in the number of CARs recorded between the two strains during training; and (2) significant differences in the number of CARs performed by Sprague-Dawley rats given DSP-4 and those given saline ($p<0.05$, Student's t-test).

was still a significant depletion of cortical NE but much less than in the control group given DSP-4 alone (Fig. 2). Brain stem NE levels were reduced to a similar extent in both strains by DSP-4 as shown in Fig. 3. It seems that DMI was once again more protective in Sprague-Dawley rats than in Fischer 344 animals (Fig. 3). One must take into account, however, that the level of NE was higher in Fischer 344 control rats than in other control groups. Treatment with DSP-4 in either strain of rat failed to lower the level of striatal dopamine (Fig. 4). In the Fischer 344 strain, the level of dopamine seen in the striata removed from animals given DMI was actually greater than the levels seen in control animals (Fig. 4).

Experiment II. Effect of DSP-4 on CAR Performance in Animals Trained in CAR

In order to study the effect of DSP-4 on CAR performance in rats trained in the CAR paradigm, DSP-4 was given to Fischer 344 and Sprague-Dawley rats which had had nine previous training sessions in CAR. The Fischer rats learned at a faster rate as indicated by the fact that they were performing significantly more CARs on Days 5–9 of training than were the Sprague-Dawley rats (Fig. 5). In trained Fischer 344 animals, there was no significant difference in CAR performance between DSP-4- and vehicle-treated rats. Furthermore, there was no decline in peformance from the pre-DSP-4 level (Fig. 5). In Sprague-Dawley rats, on the other hand, there was a significant difference in CAR responding by DSP-4- and vehicle-treated rats by the second day of testing after the DSP-4 treatment. Examination of the learning curves in Fig. 5 reveals that there was no loss in CAR performance in DSP-4-treated animals, but that further increases in performance did not occur in NE-depleted animals. The DSP-4-induced diminution in acquisition of the CAR, hinted at in the previous experiment, reached statistical

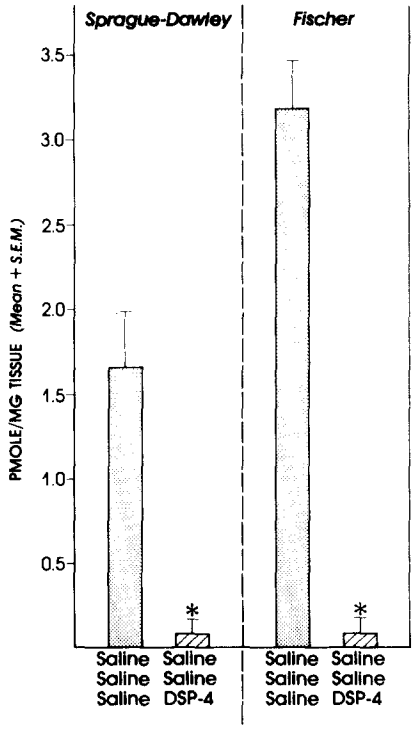

FIG. 6. Mean (+S.E.M.) levels of norepinephrine detected in the cortex of Sprague-Dawley (left) and Fischer 344 (right) rats treated with DSP-4 or saline (n=4–6 per group). In this and the next two figures, saline saline saline refers to the three injections given the control animals over the course of the experiment. In addition, the * denotes a significant difference from the control group ($p<0.05$, Student's t-test).

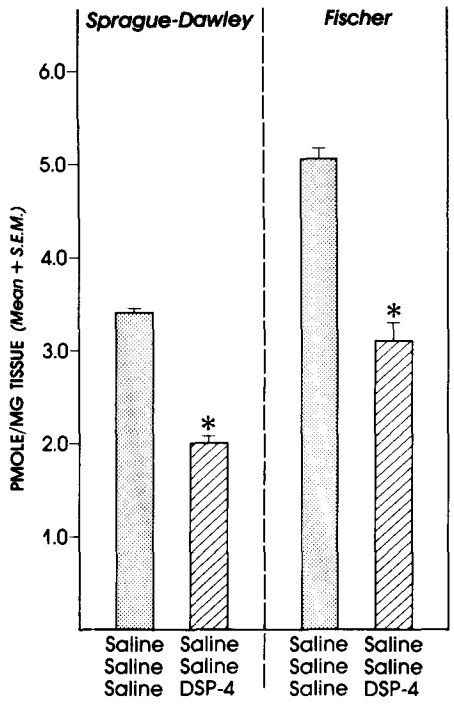

FIG. 7. Norepinephrine in brain tissue of Sprague-Dawley (left) and Fischer 344 (right) rats (n=4–6 per group). The * denotes a significant difference from the control group ($p<0.05$, Student's t-test).

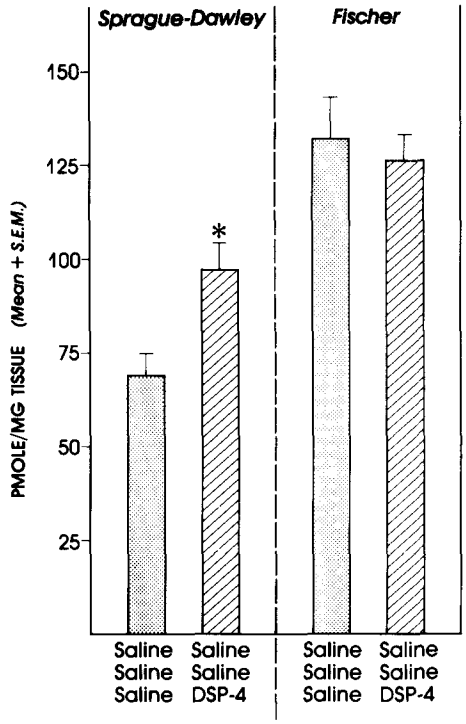

FIG. 8. Dopamine levels in striatal tissue for Sprague-Dawley (left) and Fischer 344 (right) rats (n=4–6 per group). Treatments are as indicated in Fig. 7 and 8. The * denotes a significant difference from the control level ($p<0.05$, Student's t-test).

significance in the Sprague-Dawley strain in this experiment.

Once again, NE levels in the cortex of both strains of animals were markedly reduced following DSP-4 administration (Fig. 6) and were reduced to a lesser extent in the brain stem (Fig. 7). Striatal dopamine levels were not reduced in either strain, but striatal tissue from Sprague-Dawley rats given DSP-4 contained a higher level of dopamine than striatal tissue from nontreated animals (Fig. 8).

DISCUSSION

The purpose of the present experiments was to determine whether cerebral NE neurons play a role in either the acquisition or retention of CAR in either the Sprague-Dawley or Fischer 344 rat. The results indicate NE may perhaps play a minor role in the acquisition of CAR since there was a trend for DSP-4-treated animals to require more training to learn the CAR task. The fewer CARs performed by DSP-4-treated rats relative to control animals was a significant difference in only Experiment II for the Sprague-Dawley strain although such a trend was apparent for both strains in the first experiment (Fig. 1).

The relatively minor decrement in performance, especially when the magnitude of the NE depletion is considered, may explain why there are conflicting reports in the literature as to the effect of DSP-4 on acquisition of CAR in Sprague-Dawley rats. Ögren *et al.* [15] also reported a trend for more trials to reach criterion on a CAR task in DSP-4-treated rats, whereas Bennett *et al.* [3] reported that DSP-4-treated rats were similar to control animals in acquisition of an active avoidance task. It is clear that NE is not the crucial cerebral transmitter for avoidance learning since almost total reduction of cortical NE simply results in an animal that requires a few additional trials to reach criterion in a CAR task. Moreover, the data also suggest cortical NE loss has no effect on performance of a CAR task that has been previously learned.

The present experiments were not carried out to examine the role of cerebral NE in learning as has been done by others utilizing central injections of 6-hydroxydopamine [6, 13, 14], rather they were carried out to determine what component of a discrete trial lever-press CAR paradigm might be served by cerebral NE. If an agent blocks CAR, can one surmise it has central antiadrenergic activity? One might infer from the present data that an agent with a central antiadrenergic property might produce some reduction of performance in CAR when an acquisition paradigm is used. The compound should produce no effect, however, if a paradigm employing a trained animal is utilized.

Of interest to experimenters wishing to set up a CAR test, are the results in the second experiment which show that Fischer 344 rats learn the CAR task at a faster rate than do Sprague-Dawley animals. These results confirm those of Davidson and Weidley [5]. In our laboratory, the Fischer 344 rat has been found to outperform Wistar and Long-Evans rats as well (unpublished observations). This strain would seem to be the strain of choice for setting up a CAR test.

REFERENCES

1. Archer, T. DSP4 (N-2-chloroethyl-N-ethyl-2-bromobenzylamine), a new noradrenaline neurotoxin and stimulus conditions affecting acquisition of two way active avoidance. *J Comp Physiol Psychol* **96:** 476–490, 1982.
2. Archer, T., S. O. Ögren, G. Johansson and S. B. Ross. DSP4-induced two way active avoidance impairment in rats: involvement of central and not peripheral noradrenaline depletion. *Psychopharmacology (Berlin)* **76:** 303–309, 1982.
3. Bennett, C., J. L. McGaugh, M. Arnold and K. C. Liang. Behavioral measures after central noradrenergic depletion with the neurotoxin, DSP-4: lack of impairment in avoidance tasks. *Soc Neurosci Abstr* **9:** 830, 1983.
4. Cook, L. and Weidley. Behavioral effects of some psychopharmacological agents. *Ann NY Acad Sci* **66:** 740–752, 1957.
5. Davidson, A. B. and E. Weidley. Differential effects of neuroleptic and other psychotropic agents on acquisition of avoidance in rats. *Life Sci* **18:** 1279–1284, 1976.
6. Fibiger, H. C. and S. T. Mason. The effects of dorsal bundle injections of 6-hydroxydopamine on avoidance responding in rats. *Br J Pharmacol* **64:** 601–605, 1978.
7. Herz, A. Drugs and the conditioned avoidance response. *Int Rev Neurobiol* **2:** 229–277, 1960.
8. Janssen, A. J., C. J. E. Niemegeers and K. H. L. Schellekens. Is it possible to predict the clinical effects of neuroleptic drugs (major tranquillizers) from animal data? *Arzneimittelforsch* **15:** 104–117, 1965.
9. Jonsson, G., H. Hallman, F. Ponzio and S. Ross. DSP4 [N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine]—a useful denervation tool for central and peripheral noradrenaline neurons. *Eur J Pharmacol* **72:** 173–188, 1981.
10. Kuribara, H. and S. Tadokoro. Correlation between anti-avoidance activities of antipsychotic drugs in rats and daily clinical doses. *Pharmacol Biochem Behav* **14:** 181–192, 1981.
11. Leysen, J. E., W. Gommeren and P. M. Laduron. Distinction between dopaminergic and serotonergic components of neuroleptic binding sites in limbic brain areas. *Biochem Pharmacol* **28:** 447–448, 1979.
12. Lipinski, J. F., B. M. Cohen, G. S. Zubenko and C. M. Waternaux. Adrenoceptors and the pharmacology of affective illness: a unifying theory. *Life Sci* **40:** 1947–1964, 1987.
13. Mason, S. T. and H. C. Fibiger. Noradrenaline and avoidance learning in the rat. *Brain Res* **161:** 321–333, 1979.
14. Mason, S. T. and S. D. Iversen. Learning in the absence of forebrain noradrenaline. *Nature* **258:** 422–424, 1975.
15. Ögren, S. O., T. Archer and S. B. Ross. Evidence for a role of the locus coeruleus noradrenaline system in learning. *Neurosci Lett* **20:** 351–356, 1980.
16. Richelson, E. and A. Nelson. Antagonism by neuroleptics of neurotransmitter receptors of normal human brain in vitro. *Eur J Pharmacol* **103:** 197–204, 1984.
17. Ross, S. B. Long-term effects of N-2-chloroethyl-N-ethyl-2-bromobenzylamine hydrochloride on noradrenergic neurones in the rat brain and heart. *Br J Pharmacol* **58:** 521–527, 1976.
18. Seeman, P. Brain dopamine receptors. *Pharmacol Rev* **32:** 229–313, 1981.