Pharmacology Biochemistry & Behavior, Vol. 7, pp. 191–194.

Evidence for Interactions Between Central Noradrenergic Neurons and Adrenal Hormones in Learning and Memory[1]

D. C. S. ROBERTS AND H. C. FIBIGER

Division of Neurological Sciences, Department of Psychiatry, University of British Columbia, Vancouver, B. C., V6T 1W5, Canada

(Received 28 February 1977)

ROBERTS, D. C. S. AND H. C. FIBIGER. *Evidence for interactions between central noradrenergic neurons and adrenal hormones in learning and memory.* PHARMAC. BIOCHEM. BEHAV. 7(3) 191–194, 1977. – The role of ascending noradrenergic projections in the acquisition and retention of a passive avoidance step-down response was evaluated by means of bilateral stereotaxic 6-hydroxydopamine-induced lesions of these systems. Lesions of the dorsal NA bundle alone, or in combination with lesions of the ventral NA bundle, failed to influence either the acquisition or retention of the passive avoidance response. In contrast, animals subjected to dorsal and ventral NA bundle lesions and adrenalectomy exhibited severe deficits in both the acquisition and retention of this response, and this effect was of the same magnitude as was observed after posttrial injections of diethyldithiocarbamate (DDC, 300 mg/kg). Adrenalectomy by itself had a small but significant effect on retention but did not influence acquisition of the response. The results are discussed with reference to the possibility that interactions between adrenal hormones and central NA mechanisms may serve important roles in learning and memory. However, the data provide no support for the hypothesis that central NA neurons are, by themselves, critically involved in these phenomena.

Adrenalectomy Noradrenaline 6-Hydroxydopamine Passive avoidance learning

KETY [11,12] and Crow [4,5] have separately proposed theories on the neural basis of learning. Both theories posit that the noradrenergic projection from the nucleus locus coeruleus (LC) to the hippocampus and cortex is fundamentally involved in learning and in memory consolidation. This hypothesis has received support from experiments utilizing diethyldithiocarbamate (DDC), a drug which inhibits the noradrenaline (NA) synthetic enzyme, dopamine-β-hydroxylase. Subcutaneous injections of DDC before or immediately following training result in a passive avoidance deficit in mice and rats when tested 24–72 hr later [22,28]. The fact that this effect can be reversed by intraventricular administration of NA has been taken as evidence that DDC produces its effects via central as opposed to peripheral mechanisms [28]. Furthermore, lesions of the coeruleo-telencephalic NA projection have been reported to produce deficits in learning and memory [3,6]. In marked contrast to these results, however, several other laboratories have failed to observe deficits in the acquisition and retention of a variety of conditioned responses after extensive lesions of this projection [2, 14, 15, 24]. At present therefore, if the NA projections of the LC serve any function in learning and memory, their precise role remains to be elucidated.

Recently Ogren and Fuxe [21] obtained evidence for functional interaction between the coeruleo-telencephalic NA system and the pituitary-adrenal axis during learning. These workers found that while neither adrenalectomy nor 6-hydroxydopamine (6-OHDA) lesions of the coerulo-cortical NA pathway produced a deficit in conditioned avoidance responding (CAR) by themselves, these treatments in combination markedly impaired the retention of that response. In addition, rats which had received both treatments were also completely unable to relearn the avoidance response. In the present study, the effects of central 6-OHDA injections alone, and in combination with adrenalectomies were investigated in a passive avoidance stepdown task. For comparative purposes, the amnesic property of DDC was also examined in the same experimental paradigm. Finally, inasmuch as DDC has recently been demonstrated to produce a conditioned taste aversion [23] postacquisition injections of lithium chloride (LiCl) were utilized to examine the possibility that sickness or malaise may have been sufficient to produce the DDC-induced retention deficits.

METHOD

Male Wistar rats (Woodlyn Farms, Guelph, Ontario)

[1] Supported by the Medical Research Council. D.C.S.R. is an MRC Student, H.C.F. is an MRC Scholar. We thank B. Richter for her excellent technical assistance.

weighting 300–330 g were anaesthetized with sodium pentobarbital (50 mg/kg) and prepared for surgery in a Kopf stereotaxic apparatus. One group received 6-OHDA injections aimed at the dorsal component of the ascending noradrenergic systems (DB). Bilateral stereotaxic infusions of 6-OHDA hydrobromide (4 μg/2 μl, expressed as the free base) dissolved in isotonic saline were performed via a Hamilton microsyringe at the rate of 2 μl/5 min. Coordinates from stereotaxic zero were: AP + 2.6 mm; ML ± 1.1 mm; DV + 3.7 mm. The rat's head was held in the plane used in the atlas of König and Klippel [13]. Another group of rats received bilateral infusions of 6-OHDA aimed at both the dorsal and the ventral ascending noradrenergic systems (DVB). Coordinates used for these injections were as above plus AP + 1.4; ML ± 1.3 mm; DV + 0.7 mm. A third group was bilaterally adrenalectomized (ADREX). A fourth group (DVB + ADREX) received treatments as described for both DVB and ADREX. One control group received both sham stereotaxic and adrenalectomy operations, where the adrenals were exposed but not removed. Stereotaxic operations were performed 3–4 weeks, and adrenalectomies were performed 7–10 days, prior to behavioural testing. All animals were maintained on food and water ad lib, except in the case of adrenalectomized animals which were given access to normal saline. Each rat was individually housed in a rat colony under a 12 hr light-dark cycle at 20° C.

The step-down apparatus was a 27 × 27 × 30 cm high Plexiglas box equipped with a 7.5 × 26.7 cm shelf 9.5 cm above the grid floor. A microswitch under the shelf controlled a timer and shock scrambler (Lehigh Valley) which delivered 1 mA foot shock through the grid floor. Each rat was placed on the shelf and the latency to step down was recorded. Upon releasing the microswitch by stepping off the shelf, the rat received scrambled foot shock until returning to the safe platform. The number of descents was recorded until the rat remained on the shelf continuously for 3 min, at which time it was removed from the apparatus. The total time to reach this criterion was recorded. Twenty-four hr later, each rat was again placed on the shelf and the latency to step down measured to a maximum of 180 sec. The procedure was adapted from Santos-Anderson and Routtenberg [26]. In addition to the lesioned animals, two groups of rats were included which received drug injections immediately following training. One group received diethyldithiocarbamate (DDC) (300 mg/kg, SC). The other group received LiCl (0.15 M, 2 ml/100 mg; IP).

After completion of behavioural testing, all lesioned animals were killed by cervical fracture between 9:00 and 11:00 a.m. Their brains quickly removed, and the hypothalamus, hippocampus and cortex was dissected out on ice as previously described [25]. NA was measured spectrophotofluorometrically by the method of McGeer and McGeer [17].

RESULTS

Table 1 shows that near total depletions (96–97%) of cortical NA were achieved in both the DB and DVB groups. Dorsal bundle lesions reduced hypothalamic NA by 57% while combined dorsal and ventral bundle lesions (DVB) reduced hypothalamic NA by 78%. Table 1 also shows the group means on each of the acquisition measures. No differences were observed between any of the groups on the initial latency to step off the platform (col. 2). A significant difference was observed when the group with adrenalectomies plus dorsal and ventral 6-OHDA injections (DVB–6–OHDA–ADREX) was compared to the appropriate sham operated controls on both the number of descents and time to criterion. No other group differed from their control group on any of the measures of acquisition.

TABLE 1

PASSIVE AVOIDANCE ACQUISITION AND BRAIN NA CONTENT OF THE VARIOUS TREATMENT GROUPS

		Measures of acquisition of a passive avoidance response to a criterion of 3 min			NA content of brain regions following various 6-OHDA treatments	
	N	Initial step-down latency	Total No. step-downs to criterion	Total time (sec) to reach criterion	Cortex and hippocampus NE (ng/g)	Hypothalamus NE (ng/g)
DB-6-OHDA	12	3.6 ± 1.1	3.7 ± 1.0	289.6 ± 29.1	13 ± 2 (3%)	1020 ± 181 (43%)
DVB-6-OHDA	10	5.1 ± 2.0	4.6 ± 0.6	321.9 ± 30.1	16 ± 2 (4%)	512 ± 79 (22%)
BURR HOLE	12	4.3 ± 1.3	4.0 ± 0.8	312.6 ± 27.0	408 ± 20	2360 ± 140
DVB-6-OHDA+ADREX	16	7.0 ± 2.4	5.4 ± 1.0*	440.0 ± 46.8*	18 ± 3 (4%)	420 ± 34 (18%)
BURR HOLE+ADREX	20	7.1 ± 1.2	3.4 ± 0.4	318.0 ± 45.5		
BURR HOLE+SHAM	17	4.6 ± 0.7	3.8 ± 0.4	299.2 ± 26.0		
DDC	8	6.5 ± 1.3	3.6 ± 0.8	286.4 ± 32.3		
LiCl	9	4.4 ± 0.6	3.4 ± 0.6	280.7±28.9		

*$p<0.01$, compared to Burr Hole + Sham; Student's t-test.

Data represent means (± SEM). DB-6-OHDA received bilateral 6-OHDA injections into the dorsal NA bundle. BVD-6-OHDA received two bilateral 6-OHDA injections into the dorsal and ventral NA bundles. Burr hole group were sham operated but received no injection. Some groups in addition received bilateral adrenalectomies (ADREX) or sham adrenalectomies (SHAM). Two groups of unoperated animals received either LiCl or DDC immediately following testing.

Figure 1 shows the mean (± SEM) and median step down latency for each group on the 24 hr step down test. Three groups were significantly different from controls: ADREX, DVB + ADREX, and DDC. The retest step-down latencies for DVB + ADREX was significantly shorter than the ADREX group, and significantly longer than the DDC group. By contrast, sham operations, posttraining LiCl injections, or 6-OHDA injections alone did not significantly impair the retention of the passive avoidance response.

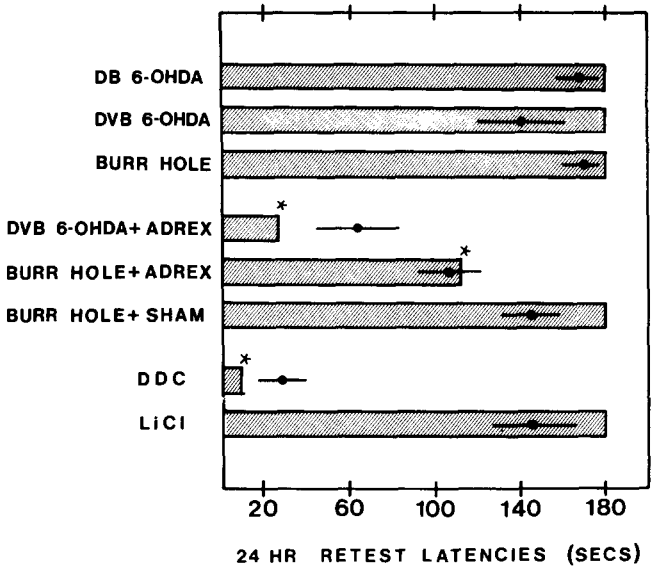

FIG. 1. Bars indicate median step-down latencies of various groups 24 hr after acquisition of a passive avoidance response. Mean (± SEM) response latencies are indicated by solid circles (•). See Table 1 and text for group abbreviations. *Significantly different from controls $p<0.05$ (Mann-Whitney U test).

DISCUSSION

Our finding that the acquisition and retention of the passive avoidance task was unimpaired in rats with near total depletion of telencephalic NA stands in contrast to the findings of Crow and Wendlandt [6]. These authors reported deficits in a passive avoidance step-down task after 6-OHDA lesions similar to those reported here. At least two major procedural differences exist, however, which may account for this discrepancy. Crow and Wendlandt [6] used four times the amount of 6-OHDA, in a concentration which may produce considerable nonspecific damage [1,10]. Since comparable telencephalic NA deplections were achieved in both studies, the deficits reported by Crow and Wendlandt [6] may have been due to nonspecific effects of their lesions unrelated to the destruction of the coeruleo-telencephalic NA projections. The studies also differ in behavioural test procedure. The animals in the present study were trained to a specific criterion. Crow and Wendlandt [6] utilized a one trial step-down procedure, and therefore may have measured retention of a partially trained response. It is possible that animals with extensive telencephalic NA depletion show a memory impairment when the response is not fully learned. In any event, it is clear that rats with very extensive lesions of the ascending NA projections to the telecephalon can learn and retain a passive avoidance response. These projections do not, therefore, appear to be critical neural substrates for learning and memory [2, 14, 15, 24].

The group which sustained bilateral adrenalectomies did show a significantly shorter retest step-down latency. This effect was unexpected. Weiss *et al.* [29] have reported that adrenalectomy does not affect a passive avoidance step-through task, and Moyer [19] has reported no effects of adrenalectomy on the acquisition and extinction of an escape response. In contrast, Dupont *et al.* [7] have shown that plasma corticosterone concentrations correlate positively with passive avoidance learning, an effect consistent with the present data. Also, Endrőczi [8] has demonstrated corticosterone injections facilitate passive avoidance behaviour in adrenalectomized rats. The tasks employed, however, differ in each of these reports. It appears, therefore, that the effects of adrenalectomy on avoidance learning may depend upon the behavioural requirements specified by different experimental paradigms.

The major finding in the present experiment was that lesions of central NA neurons in combination with adrenalectomy caused profound deficits in both the acquisition and retention of a passive avoidance response. These animals stepped off the platform significantly more times during the initial learning phase of the experiment, and a significantly longer time was required to reach criterion. On the 24 hr retest, this group showed a significant memory impairment as measured by a shorter latency to step off the platform. Since all animals were trained to the same criterion, this shorter latency indicates a retention impairment in addition to the acquisition deficit observed during learning. These results complement those of Ögren and Fuxe [21], who reported severe impairments in the acquisition and retention of a conditioned avoidance response in animals which had received both 6-OHDA lesions of the dorsal NA bundle and adrenalectomies. Both these and the present observations point to an interaction between the pituitary-adrenal axis and central NA mechanisms which is important in learning and memory. At present, the nature and anatomical location of this interaction are unknown. Furthermore, to what extent loss of hormones from the adrenal cortex as opposed to the adrenal medulla may participate in these effects is an important question concerning which there is currently no information. It is of interest, however, that the DVB + ADREX group approached the same degree of retention deficit as was observed in the DDC group. This raises the possibility that the retention deficit produced by DDC is mediated via a combined inhibition of NA synthesis in both the periphery and the central nervous system. In this regard, it is noteworthy that either central or peripheral administration of NA can effectively reverse DDC-induced retention deficits [18]. On the other hand, the fact that corticosterone has recently been shown to increase the conversion of tyrosine to NA in brain [9] raises the possibility that the loss of certain interactions between adrenal corticoids and cental NA neurons mediated the impaired acquisition retention in the DVB–ADREX group. In this regard, perhaps it is not without significance that corticosterone has been shown to bind to the hippocampus [16], a structure which is innervated by the dorsal NA bundle [30]. Additional studies will be required to discriminate between these alternatives.

Using the conditioned taste aversion (CTA) paradigm, DDC has recently been shown to have significant punishing properties [23]. This raises the possibility that the amnesic effects of DDC may have been mediated at least in part by

its aversive properties. The LiCl results clearly argue against this interpretation because a dose of LiCl which produces a CTA of equal or greater magnitude than that induced by DDC (300 mg/kg) [20,23], had no effect upon retention of the passive avoidance response. These results are similar to recent findings by Squire *et al.* [27] who concluded that the amnesia induced by protein synthesis inhibitors could not be explained by their aversive properties.

In summary, the present observations failed to support the hypothesis that ascending central NA projections are, by themselves, critical neural substrates for learning and memory. Lesions of the ventral and dorsal NA bundles affected neither the acquisition nor retention of a passive avoidance response. In contrast, a combination of adrenalectomy and 6-OHDA lesions of the ascending NA projections produced severe acquisition and retention deficits. These observations support previous suggestions that interactions between the pituitary-adrenal axis and central NA neurons may be involved in some types of learning and memory.

REFERENCES

1. Agid, Y., F. Javoy, J. Glowinski, D. Bovet and C. Sotelo. Injection of 6-hydroxydopamine into the substantia nigra. II Diffusion and specificity. *Brain Res.* **58:** 291–301, 1973.
2. Amaral, D. G. and J. A. Foss. Locus coeruleus lesions and learning. *Science* **188:** 377–378, 1975.
3. Anlezark, G. M., T. J. Crow and A. P. Greenway. Impaired learning and decreased cortical norepinephrine after bilateral locus coeruleus lesions. *Science* **181:** 682–684, 1973.
4. Crow, T. J. Cortical synapses and reinforcement: A hypothesis. *Nature,* Lond. **219:** 736, 1968.
5. Crow, T. J. and G. W. Arbuthnott. Function of catecholamine-containing neurons in mammalian central nervous system. *Nature* **238:** 245–246, 1972.
6. Crow, T. J. and S. Wendlandt. Impaired acquisition of a passive avoidance response after lesions induced in the locus coeruleus by 6–OH–dopamine. *Nature,* Lond. **259:** 42–44, 1976.
7. DuPont, A., E. Endróczi and C. Fortier. Relationship of pituitary-thyroid and pituitary-adrenocortical activities to conditioned behavior in the rat. In: *Influence of Hormes on the Nervous System,* edited by D. H. Ford. Basel: Karger, 1971, pp. 451–462.
8. Endróczi, E. Pituitary-adrenocortical activity, exploration and avoidance behavior in the rat. In: *Steroid Hormones and Brain Function,* edited by C. H. Sawyer and R. A. Gorski. Berkeley: University of California Press, 1971, pp. 59–66.
9. Iuvone, P. M., R. L. Delanoy and A. J. Dunn. Effects of pituitary-adrenal hormones on catecholamine synthesis in mouse brain. *Neuroscience Abstracts* **2:** 786, 1976.
10. Javoy, F. Partial selectivity of 6–OHDA induced neuronal degeneration after intratissular injection in the brain with special reference to the nigro-striatal dopamine system. *J. Neurol. Trans.* **37:** 219–227, 1975.
11. Kety, S. S. The biogenic amines in the central nervous system: The possible roles in arousal, emotion, and learning. In: *The Neuroscience: Second Study Program,* edited by F. O. Schmitt. New York: Rockefeller University Press, 1970, pp. 324–336.
12. Kety, S. S. The possible role of the adrenergic systems of the cortex in learning. *Res. Publs Ass. Res. nerv. ment. Dis.* **50:** 376–389, 1972.
13. König, J. F. and R. A. Klippel. *The Rat Brain: A Stereotaxic Atlas.* Baltimore: Williams and Wilkins, 1963.
14. Mason, S. T. and S. D. Iversen. Learning in the absence of forebrain noradrenaline. *Nature* Lond. **258:** 422–424, 1975.
15. Mason, S. T. and S. D. Iversen. The effects of selective forebrain noradrenaline loss on tests of behavioural inhibition. *J. comp. physiol. Psychol.* **91:** 165–173, 1977.
16. McEwan, B. S. and D. W. Pfaff. Chemical and physiological approaches to neuroendocrine mechanisms: Attempts at integration. In: *Frontiers in Neuroendocrinology,* edited by W. F. Ganong and L. Martini. New York: Oxford University Press, 1973, pp. 267–335.
17. McGeer, E. G. and P. L. McGeer. Catecholamine content of the spinal cord. *Can. J. Biochem. Physiol.* **40:** 1141–1151, 1962.
18. Meligeni, J. A., S. A. Ledergerber and J. L. McGaugh. Reversal of diethyldithiocarbamate induced amnesia by norepinephrine. *Neuroscience Abstracts* **1:** 514, 1975.
19. Moyer, K. E. Effect of adrenalectomy on anxiety motivated behaviour. *J. gen. Psychol.* **92:** 11–16, 1958.
20. Nachman, M. Learned taste and temperature aversions due to lithium chloride sickness after temporal delays. *J. comp. physiol. Psychol.* **73:** 22–30, 1970.
21. Ögren, S. –O. and K. Fuxe. Learning, brain noradrenaline and the pituitary-adrenal axis. *Med. Biol.* **52:** 399–405, 1974.
22. Randt, C. T., D. Quarterman, M. Goldstein and B. Anagnoste. Norepinephrine biosynthesis inhibition: Effects on memory in mice. *Science* **172:** 498–499, 1971.
23. Roberts, D. C. S. and H. C. Fibiger. Conditioned taste aversion induced by diethyldithiocarbamate (DDC), *Neuroscience Letters* **2:** 339–342, 1976.
24. Roberts, D. C. S., M. T. C. Price and H. C. Fibiger. The dorsal tegmental noradrenergic projection: An analysis of its role in maze learning. *J. comp. physiol. Psychol.* **90:** 363–372, 1976.
25. Roberts, D. C. S., A. P. Zis and H. C. Fibiger. Ascending catecholamine pathways and amphetamine-induced locomotor activity: Importance of dopamine and apparent non-involvement of norepinephrine. *Brain Res.* **93:** 441–454, 1975.
26. Santos-Anderson, R. M. and A. Routtenberg. Stimulation of rat medial or sulcal prefrontal cortex during passive avoidance learning selectively influences retention performance. *Brain Res.* **103:** 243–259, 1976.
27. Squire, L. R., C. A. Emanuel, H. P. Davis and J. A. Deutsch. Inhibitors of cerebral protein synthesis: Dissociation of aversive and amnesic effects. *Behav. Biol.* **14:** 335–341, 1975.
28. Stein, L., J. D. Belluzzi and C. D. Wise. Memory enhancement by central administration of norepinephrine. *Brain Res.* **84:** 329–335, 1975.
29. Weiss, J. M., B. S. McEwen, M. T. A. Silva and M. F. Kalkut. Pituitary-adrenal influences on fear responding. *Science* **163:** 197–199, 1969.
30. Ungerstedt, U. Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta physiol. scand.* **367:** 1–48, 1971.