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Possible Role of Dopamine-β-hydroxylase in the Regulation of Norepinephrine Biosynthesis in Rat Brain

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WISE, C. D., BELLUZZI, J. D. AND L. STEIN. *Possible role of dopamine-β-hydroxylase in the regulation of norepinephrine biosynthesis in rat brain.* PHARMAC. BIOCHEM. BEHAV. 7(6) 549–553, 1977. – In Experiment 1, the dose-response effects of three dopamine-β-hydroxylase (DBH) inhibitors (diethyldithiocarbamate, FLA-63 and U-14, 624) on the endogenous levels of norepinephrine and dopamine in pons-medulla of rat brain were determined. In Experiment 2, the effect of low doses of diethylithiocarbamate (2.5 to 120 mg/kg) on the level of norepinephrine-^{3}H produced from dopamine-H^3 was determined. The data obtained by extrapolation of the curves in both experiments provided an estimation of the in vivo level of DBH activity and suggested that it was not present in excess. Finally, in Experiment 3, the three DBH inhibitors reduced self-stimulation (a behavior dependent upon catecholamines) in a dose-related manner and intraventricular injections of l-norepinephrine reinstated normal rates of self-stimulation. The results from the three experiments are consistent with the idea that DBH is involved in the regulation of norepinephrine biosynthesis. The relationship of this finding to our earlier report of a deficit of DBH in post-mortem brains of schizophrenics is discussed.

Norepinephrine Dopamine Norepinephrine biosynthesis Dopamine-β-hydroxylase
Self-stimulation Schizophrenia

IN SOME peripheral tissues, norepinephrine (NE) biosynthesis may be regulated largely, if not exclusively, at the tyrosine hydroxylase step [13, 16, 19, 25]. It is commonly assumed that sole regulation at the tyrosine hydroxylase step similarly controls NE biosynthesis in the brain (see discussion [17]). However, the regulatory step in a metabolic sequence frequently may vary in different tissues or even in the same tissue in different species, as Kaufman and Friedman [13] have pointed out. According to these workers, the facts are not all consistent with the conclusion that tyrosine hydroxylase exclusively regulates NE synthesis in the brain. Indeed, this conclusion is not consistent with much recent anatomical [27], neurochemical [8, 15, 17, 18], and psychopharmacological data [30].

The anatomical work reveals that in certain typically noradrenergic brain regions, such as locus coeruleus, the concentration of dopamine (DA) is at least twice as high as that found in peripheral tissues [1]. Although these relatively high DA levels might be due to presently unknown DA cell bodies or terminals, it is also possible that, due to regulation of NE synthesis at the DBH step, DA could be present in excess in the NE neurons.

The neurochemical studies show that the brain contains high concentrations of endogenous inhibitors [8, 15, 17, 18]. It has been suggested that some of these inhibitors may be involved in the physiological regulation of DBH activity in vivo. Lander and Austin [15] have shown that the caudate nucleus contains relatively high amounts of DBH (77 to 89% of the DBH activity in hypothalamus) but relatively low levels of NE (11% of that found in the hypothalamus [5]). Significant amounts of a sulfhydryl inhibitor were associated with the synaptic vesicle fraction. Lander and Austin further reported that free cupric ion concentrations in hypothalamus and caudate nucleus exceeded that required to inhibit DBH in vitro, and that there was a higher percentage of free copper in caudate nucleus than in hypothalamus. They propose "that NA (norepinephrine) synthesis in various regions of brain is also regulated at the DBH step by both the sulfhydryl inhibitor and cupric ions" [15].

Finally, psychopharmacological studies suggest that self-stimulation behavior, which depends heavily on NE transmission, is sensitive even to low doses of DBH inhibitors [30]. If DBH were present in great excess, the behavior should be relatively insensitive to these small doses.

Taken together, the foregoing evidence suggests the possibility that brain NE biosynthesis may not only be regulated at the tyrosine hydroxylase step, but at least in some circumstances at the DBH step as well. We report here pharmacological, biochemical, and behavioral evidence that provides additional support for this idea.

EXPERIMENT 1

NE-depleting effects of several drugs that inhibit DBH were determined in a brain region, the pons-medulla, that is reported to contain few or no DA neurons [26]. If DBH were critically involved in the regulation of NE biosyn-

thesis, the DBH inhibitors would produce dose-related decreases in the level of NE, and even small doses would be effective. In the best case, extrapolation of the dose-response curve to zero dose would yield the same NE value as that for undrugged controls. On the other hand, if the enzyme were present in excess, all drug doses below that necessary to inhibit the excess DBH would be ineffective. In such case, the dose-response curve would be discontinuous and displaced to the right.

METHOD

Male Charles River rats (150–200 g) were deprived of food overnight. Groups of 4 to 6 rats per dose of drug were injected with saline or different doses of DBH inhibitors (diethyldithiocarbamate [DDC], 1 phenyl-3-(2-thiazolyl)-2-thiourea [U-14, 624], 5-butylpicolinic acid [fusaric acid] or bis-4(4-methyl-1-homopioerazinyl-thiocarbonyl)-disulphide [FLA-63]. The compounds were dissolved in water or, if necessary, suspended in water that contained 0.5% Tween 80. DDC was administered subcutaneously and the other drugs were given intraperitoneally (IP). Three (DDC and fusaric acid) or 4 (FLA-63 and U-14, 624) hr later the animals were killed by decapitation and the brain quickly removed. A pons-medulla region, caudal to the inferior colliculi, was dissected out and placed in plastic bags that were then immersed in liquid nitrogen. The brain tissue was stored at −15° overnight and then analyzed the next day for NE and DA as previously described [4]. The data from the drugged animals are plotted as a percent of the saline controls, whose mean NE content was 561 ± 44.0 ng/g of tissue (wet weight) and mean DA content was 39 ± 5.3 ng/g tissue.

RESULTS

DDC and U-14, 624 produced dose-related decreases in the level of NE and proportionate increases in that of DA (Fig. 1). Plots of the NE and DA levels for each drug dose displayed high inverse correlations (DDC, −0.98 and U-14, 624, −0.88). NE levels decreased maximally to 40–50% of control values at 400 mg/kg of DDC or 20 mg/kg of U-14, 624; higher doses in neither case had little further effect. Somewhat different results were obtained with fusaric acid; although there was a substantial NE depletion, the curve did not extrapolate to near 100%. With FLA-63, doses of 2.5 and 15 mg/kg produced similar 30% decreases in NE levels. Since approximately half of the animals died after receiving 15 mg/kg of FLA-63, higher doses were not investigated. In mouse brain, however, higher doses of FLA-63 produce further decreases [24].

DISCUSSION

NE depletion induced by three of the inhibitor drugs apparently was due to DBH inhibition since there was a proportionate increase in DA levels. Furthermore, even small doses of DDC and U-14, 624 were efficacious and the depletion curves could be extrapolated to a value near 100%. These results in brain contrast with those obtained in ileum [6], where doses of DDC up to 100 mg/kg produced no decrease in NE; transmitter depletion in the ileum followed a discontinuous curve which was displaced to the right. It thus would appear that DBH is not critically involved in the regulation of NE synthesis in the rat ileum, consistent with findings for other peripheral tissues, such as heart [16].

The findings with fusaric acid appear inconsistent with those obtained with DDC and U-14, 624. One possibility is that fusaric acid effectively penetrates the brain only at higher doses. Peripherally-active decarboxylase inhibitors also are effective in the brain only at high doses and produce depletion curves that are similarly displaced to the right [3].

EXPERIMENT 2

It may be argued that the doses of DDC used in Experiment 1 were too high for the proposed analysis, and that doses below 100 mg/kg might have been ineffective. Using a sensitive radioisotope method, we therefore examined the NE-depleting effects of DDC in the dose ranges 2.5 mg/kg to 120 mg/kg.

METHOD

Rats under light ether anesthesia were injected intracisternally with 0.7 μCl (14 ng) of 3,4-dihydroxyphenylethylamine [ethyl-1-^{3}H (N)] obtained from New England Nuclear. The dopamine-H^3 was dissolved in 10 μl of Ringer-Locke solution (Ca^{++} omitted, pH 7.4). Fifteen minutes later, groups of rats were injected subcutaneously with saline or different doses of DDC. One hour after drug administration, the animals were killed and the pons-medulla was dissected out and frozen. The radioactive catecholamines were extracted and then separated on alumina and ion exchange columns according to the method described in Stein *et al.* (in preparation). The mean radioactivity of pons-medulla NE in the saline group was 867 ± 54.7 dpm/g tissue.

RESULTS

Doses of DDC as small as 2.5 mg/kg produced significant depletion of brain NE ($p<0.05$) (Fig. 2). Interestingly, the curve generated by these low doses was curvilinear, which may explain why the DDC curve in Experiment 1 extrapolated linearly to a value below 100%.

DISCUSSION

The sensitivity of the radioisotope method allowed the detection of the effects of very small doses of the DBH inhibitor. As in the first experiment, all doses of the drug used were effective in depleting NE. The results in both experiments are thus consistent with the interpretation that DBH is involved in the regulation of brain NE synthesis.

EXPERIMENT 3

If DBH were a regulatory step in NE biosynthesis, even small doses of a DBH inhibitor should disrupt behavior that is dependent on noradrenergic transmission, e.g. hypothalamic self-stimulation [2, 10, 11, 23, 29, 30]. To establish that the behavioral decrement induced by the DBH inhibitors in fact was due to NE depletion, an attempt was made to reinstate the suppressed behavior by central administration of exogenous NE.

METHOD

Twenty-eight male Charles River rats weighing 300–400

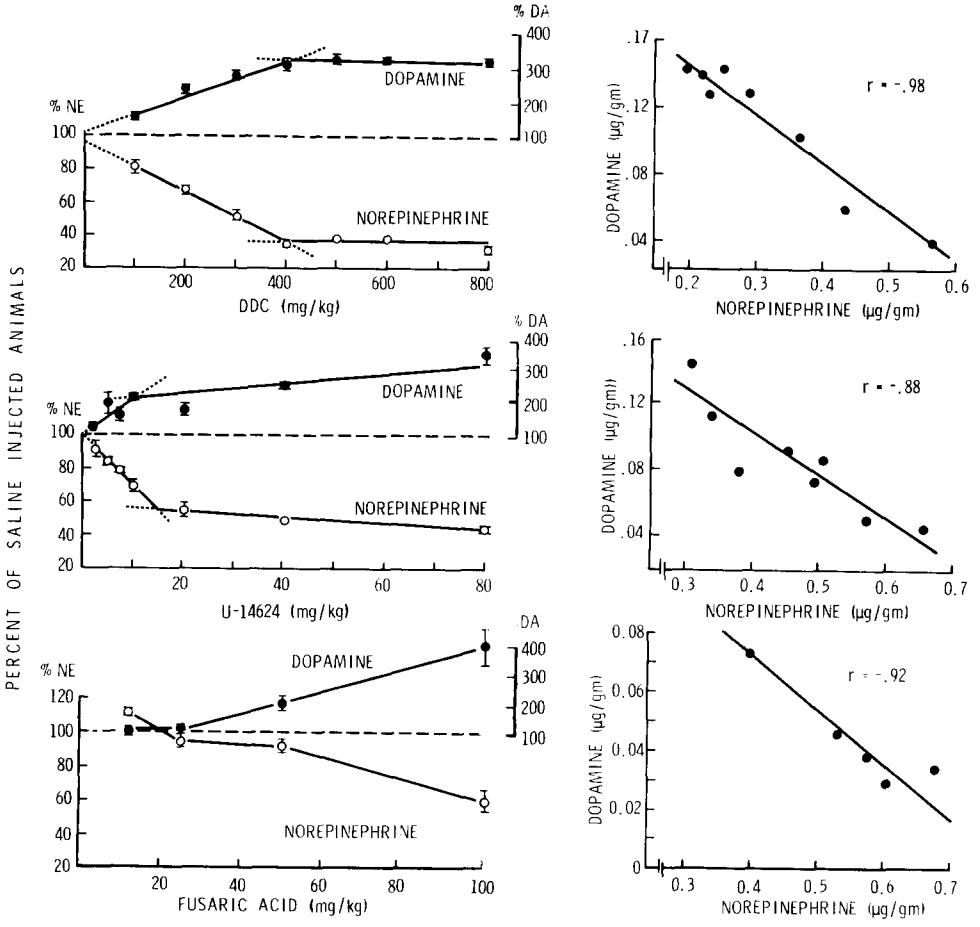

FIG. 1. The curves on the left show the effect of dopamine-β-hydroxylase inhibition by DDC, U-14, 624 and fusaric acid on norepinephrine and dopamine levels in the pons-medulla of the rat brain. Drugs were administered (4 to 6 rats per dose) systemically 3 or 4 hours before the animals were killed. In the saline or non-drugged rats, the average norepinephrine content was 561 ± 44.0 ng/gm of tissue and the dopamine content was 39 ± 5.3 ng/gm. On the right, the data are replotted to obtain the correlation coefficients.

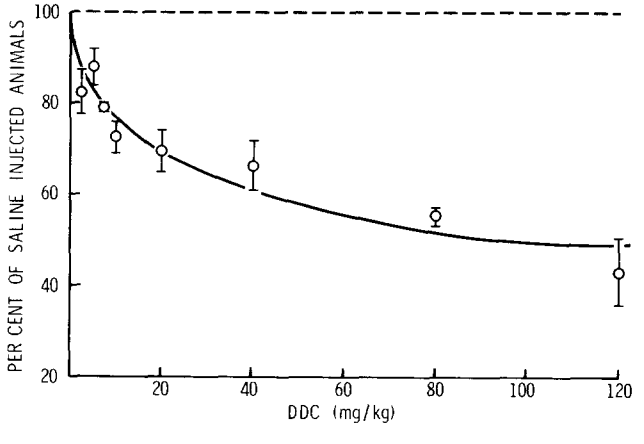

FIG. 2. Effect of low doses of DDC (2.5 to 120 mg/kg, IP) on the level of norepinephrine-^{3}H (in the pons-medulla) produced from intracisternally injected dopamine-^{3}H, 15 min after the isotope, the DDC was administered and the rats were killed 1 hr later.

g were stereotaxically implanted with 75-μm platinum-iridium electrodes in the medial forebrain bundle (MFB) at the level of the ventromedial hypothalamus. Self-stimulation methods and parameters of stimulation have been described [21]. The current was adjusted in each case to the lowest intensity that maintained a stable rate of self-stimulation (range, 500–9100 responses per hour). Rats were trained in 2-hr sessions until stable baselines were established. Separate groups of 4 to 6 rats, matched for mean self-stimulation rate, were used to evaluate the following DBH inhibitors: DDC, U-14, 624, fusaric acid and FLA-63. At intervals of 1 to 2 weeks, a different drug dose was administered as in Experiment 1, one hour before the 2-hr test.

In the NE-reversal experiment, a different group of 13 rats with an MFB electrode and lateral ventricle cannula were trained until stable rates of self-stimulation were achieved (range, 612 to 10,575 responses per hour). Each rat then received either fusaric acid (150 mg/kg), U-14,624 (75 mg/kg) or DDC (300 mg/kg) one hr before the behavioral test. During the two-hr session, each rat was aroused by handling and then returned to the chamber to

evaluate the effects of such arousal. Later in the test, at appropriately spaced intervals, intraventricular injections of Ringer-Locke vehicle (10 μl), l-norepinephrine (10 μg), and in some cases DA (10 μg) were administered.

RESULTS

DDC, U–14,624, and fusaric acid caused dose-related decreases in the self-stimulation rate (Fig. 3). FLA–63 had only a small erratic effect, but because half of the animals died after the first two drug administrations, this drug was discontinued.

l–Norepinephrine largely reinstated normal rates of self-stimulation within 15 min after its central administration in rats pretreated with DDC, U–14,624 or fusaric acid (Table 1). Specificity was suggested by the fact that handling, Ringer-Locke solution, and dopamine had negligible effects.

DISCUSSION

Restoration of normal self-stimulation rates by intraventricular NE after their suppression by DBH inhibitor treatments suggests that the inhibitor drugs exerted their behavioral action by depletion of NE and not by some other action unrelated to NE biosynthesis. This result confirms our previous work [30] and extends the observation to include two new DBH inhibitors, U-14, 624 and fusaric acid. Although FLA-63 did not effectively suppress self-stimulation in our experiments, Franklin and Herberg [10] found this drug effective if reserve pools of catecholamines were first depleted by reserpine.

The self-stimulation deficits induced by moderate doses of DDC, U-14, 625 and fusaric acid provide evidence at the behavioral level that DBH may be involved in the regulation of NE biosynthesis. However, all drugs decreased self-stimulation more extensively on a percentage basis than they did the endogenous levels of pons-medulla NE. The apparently greater sensitivity of the behavioral indicator might reflect nonspecific effects of the drugs, or it could be explained by biochemical and anatomical considerations. In the first place, measurements of total NE probably do not accurately reflect the content of the relatively small functional pools of transmitter, which are mainly responsible for the maintenance of self-stimulation behavior [30]. Secondly, to influence self-stimulation, the drugs probably act at rostral brain sites (where NE nerve-endings are

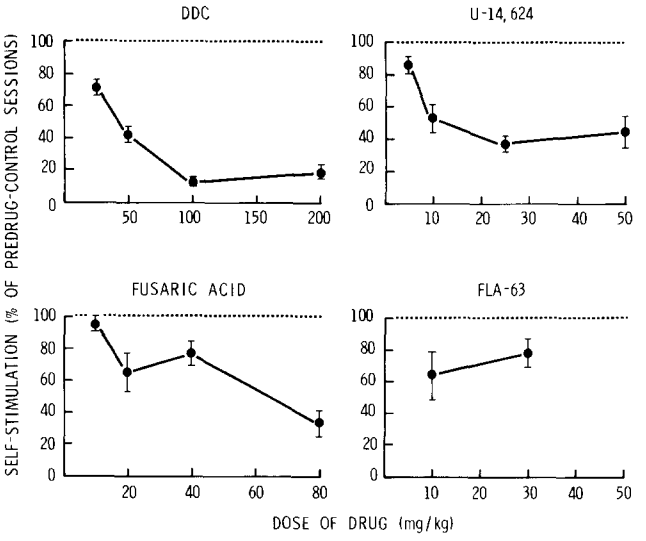

FIG. 3. Effect of DBH inhibitors on self-stimulation behavior. Drugs (4 to 6 rats per dose) were administered systemically one hr before the two hr self-stimulation session. The rates for the two hr session were compared to the average rate of the preceding 3 control days.

TABLE 1

THE EFFECT OF NOREPINEPHRINE SYNTHESIS INHIBITION AND NOREPINEPHRINE REPLETION ON SELF-STIMULATION BEHAVIOR

Treatment	Dose	Number of Rats	Mean Self-Stimulations (% of control)
Fusaric Acid	150 mg/kg		
Handling		9	39.65 ± 12.02*
Ringer-Locke		8	41.02 ± 12.95*
Norepinephrine	10μg	9	109.33 ± 27.43
Dopamine	10 μg	8	12.74 ± 4.51*
U-14,624	75 mg/kg		
Handling		11	36.17 ± 12.53
Ringer-Locke		10	23.41 ± 7.59*
Norepinephrine	10 μg	11	86.81 ± 25.48
Dopamine	10 μg	10	20.92 ± 11.36*
Diethyldithiocarbamate	300 mg/kg		
Handling		7	27.63 ± 4.39*
Ringer-Locke		9	28.79 ± 7.57*
l-Norepinephrine	10 μg	9	116.98 ± 34.50

*Significantly different from norepinephrine, $p<0.05$

concentrated) rather than at pons-medulla sites (where NE cell bodies are concentrated).

GENERAL DISCUSSION

Knowledge of the mechanisms that regulate the biosynthesis of NE obviously is required for complete understanding of noradrenergically-mediated behavioral and physiological functions. The present results support suggestions [15,17] that brain NE biosynthesis may be regulated at the DBH as well as at the tyrosine hydroxylase step. Since tyrosine hydroxylase has a much lower activity than DBH, it would seem that such regulation must be attributed to compartmentalization of the enzyme and/or to the action of endogenous DBH inhibitors.

Deficits in DBH activity ranging from 30 to 50%, depending on the region examined, were found in post-mortem specimens from schizophrenic brains [28,31]. However, only if DBH were involved in the regulation of norepinephrine synthesis could such partial deficits produce a deficiency of transmitter and consequent behavioral disorder. This possibility seems to be supported by the present observations.

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