Pharmacology Biochemistry & Behavior, Vol. 19, pp. 905–907, 1983.

BRIEF COMMUNICATION

6-Hydroxydopamine and 5,7-Dihydroxytryptamine Selectively Reduce Dopamine and 5-Hydroxytryptamine Metabolites in Cerebroventricular Perfusates of Rats

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Received 22 June 1983

NIELSEN, J. A. AND K. E. MOORE. *6-Hydroxydopamine and 5,7-dihydroxytryptamine selectively reduce dopamine and 5-hydroxytryptamine metabolites in cerebroventricular perfusates of rat.* PHARMACOL BIOCHEM BEHAV **19**(5) 905–907, 1983.—The efflux into the lateral cerebral ventricles of metabolites of dopamine (DA) and 5-hydroxytryptamine (5HT) was determined in unanesthetized rats bearing chronically implanted push-pull cannulae. Pretreatment with 6-hydroxydopamine (6-OHDA) reduced the basal efflux of 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), but not of 5-hydroxyindoleacetic acid (5HIAA). The haloperidol-induced increase in the efflux of DOPAC and HVA was markedly attenuated in the 6-OHDA-pretreated rats. In rats treated with 5,7-hydroxytryptamine (5,7-DHT) the basal efflux of DOPAC and HVA was unaffected, while that of 5HIAA was markedly reduced; in these animals the ability of L-tryptophan to increase the perfusate content of 5HIAA was abolished. These results indicate that metabolites of DA and 5HT appearing in cerebroventricular perfusates of rats originate from DA and 5HT neuronal terminals in the brain.

6-Hydroxydopamine 5,7-Dihydroxytryptamine Dopamine metabolites 5-Hydroxytryptamine metabolite Brain perfusion

THERE have been numerous reports of studies in which the activities of catecholamine and 5-hydroxytryptamine (5HT)-containing neurons in the central nervous system have been estimated from the concentrations of metabolites of these neurotransmitters in cerebroventricular perfusates. Most of these early studies employed radioactive tracers [13] or used anesthetized animals [1]. A procedure in which the efflux of endogenous metabolites of dopamine (DA) (3,4-dihydroxyphenylacetic acid, DOPAC; 3-methoxy-4-hydroxyphenylacetic acid, homovanillic acid, HVA) and of 5HT (5-hydroxyindoleacetic acid, 5HIAA) is quantified in sequential samples of perfusate collected from a push-pull cannula permanently implanted in a lateral cerebroventricle of an unanesthetized rat has recently been described [11]. Results of pharmacological studies employing this technique suggest that the efflux of the amine metabolites reflect the activity of DA and 5HT neurons. That is, haloperidol increases [6] and apomorphine decreases [5] impulse traffic in the major ascending DA neurons, and they produce corresponding changes in the efflux of DA metabolites [11]. In addition, L-tryptophan, which increases the synthesis of 5HT [7] selectively increases the perfusate concentration of 5HIAA [11]. It is generally assumed that amine metabolites collected in the cerebroventricular perfusate originate from neurons terminating in close proximity to the cerebroventricles. To test this directly, the efflux of 5HT and DA metabolites was quantified in cerebroventricular perfusates of rats pretreated with neurotoxins which selectively destroy catecholaminergic (6-hydroxydopamine, 6-OHDA) or 5HT neurons (5,7-dihydroxytryptamine, 5,7-DHT) [3].

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TABLE 1

DOPAC, HVA AND 5-HIAA IN VENTRICULAR PERFUSATE OF RATS FOLLOWING 6-OHDA (200 μg/RAT, ICV) TREATMENT AND HALOPERIDOL (1.0 mg/kg, SC) CHALLENGE

	Before 6-OHDA	6-OHDA ×1	6-OHDA ×2
DOPAC			
Before Haloperidol	11.5 ± 2.2	4.9 ± 1.7†	2.2 ± 0.8†
After Haloperidol	18.9 ± 5.2	6.7 ± 1.8	3.3 ± 0.6
Change	7.4 ± 4.1*	1.8 ± 0.5*	1.1 ± 0.4*
HVA			
Before Haloperidol	5.2 ± 0.8	2.1 ± 0.6†	1.3 ± 0.3†
After Haloperidol	8.9 ± 2.6	3.8 ± 1.0	1.9 ± 0.4
Change	3.7 ± 2.0*	1.7 ± 0.6*	0.6 ± 0.3
5-HIAA			
Before Haloperidol	5.0 ± 1.3	7.0 ± 1.4†	5.9 ± 1.3
After Haloperidol	3.6 ± 0.7	6.5 ± 1.9	5.9 ± 1.2
Change	−1.4 ± 1.1	−0.5 ± 1.9	0 ± 0.3

Values represent ng metabolites/ml perfusate (Mean ± 1 S.E., N=6) in samples collected before haloperidol (15–30 min of the perfusion session) and after haloperidol (60–75 min after the perfusion began or 30–45 min after haloperidol).

*Haloperidol-induced change is significant ($p<0.05$).

†6-OHDA-induced change is significant ($p<0.05$).

METHOD

Male Sprague-Dawley rats (Spartan Farms, Haslett, MI) weighing 225–250 g were anesthetized with Equithesin (3 ml/kg, IP) and implanted with cannula bases such that the tip of the needle tubing was within the right lateral cerebroventricle. The methods for fabricating the push-pull cannulae, of implanting the bases, and perfusing the ventricle with artificial cerebrospinal fluid have been described in detail previously [10,11]. At least one week after surgery, an unanesthetized rat was placed in a large plastic bowl and the push-pull cannula cap screwed into the implanted base. The lateral cerebral ventricle was then perfused at a rate of 20 μl/min for 75 min. Sequential samples of perfusate were collected every 15 min into microsample tubes containing 400 μl of high performance liquid chromatography mobile phase (see below); samples were frozen at −20° until analyzed.

A group of 6 rats was injected with haloperidol (1 mg/kg, SC) 30 min into a perfusion session (i.e., after collection of 2 samples), and then 3 additional 15 min samples of perfusate were collected. Approximately 2 hr after stopping the perfusion, 6-OHDA hydrobromide (200 μg of the free base in 10 μl 0.9% NaCl containing 10 mg % ascorbic acid) was infused over 10 min into the lateral cerebral ventricle of each rat. The same procedure was repeated 7 days later and the animals were again perfused and injected with haloperidol after another 7 days. A second group of 4 rats was injected IP with L-tryptophan (100 mg/kg) 30 min into a perfusion session and then 3 additional 15 min samples of perfusate were collected. This was followed in about 2 hr by an intracerebroventricular infusion (10 min) of 5,7-DHT creatinine sulfate (200 μg of the free base in 10 μl 0.9% NaCl containing 10 mg % ascorbic acid). The animals were perfused and injected with L-tryptophan 10 days later. After their last perfusion half of each group of animals were perfused via cardiac puncture with 10% neutral buffered formalin. Serial brain sections were cut at 10 μ, stained with hematoxylin and eosin, and examined for tissue damage.

Each perfusate sample was filtered and then 500 μl were injected directly onto a 30 cm×3.9 mm i.d. μBondapak C_{18} column (Waters Associates). A TL-3 carbon paste (CP-O) amperometric detector (Bioanalytical Systems) was set at +0.75V versus an $Ag^+/AgCl$ reference electrode. The mobile phase consisted of 0.1 M citrate-phosphate buffer (pH 3.0) plus 8% methanol and 0.024% sodium octyl sulfate (Eastman Kodak) (for additional details see [11]).

The amount of DOPAC, HVA and 5HIAA in each perfusate sample was determined by measuring peak heights and comparing these with standards which were analyzed under identical conditions on the same day. The sensitivity of the assay for the metabolites varied inversely with the age of the column, but 1.5 ng HVA, 0.3 ng DOPAC and 0.2 ng 5HIAA/ml of perfusate could be easily detected. Effects of treatments were evaluated using the Wilcoxon T statistic [8]; a critical value of 0.05 was set as that required to indicate a statistically reliable effect of the experimental manipulation.

RESULTS AND DISCUSSION

The basal efflux of 5HT and DA metabolites from the cerebroventricular system exhibits a great deal of variation from animal to animal, but within each animal the concentrations of these metabolites in the perfusate remain remarkably constant from day to day [11]. The concentrations of amine metabolites in the first 15 min sample is generally higher and more variable than that in subsequent samples; therefore, in Fig. and Table 1, values for the first sample are not reported. The 5HIAA, DOPAC and HVA concentration

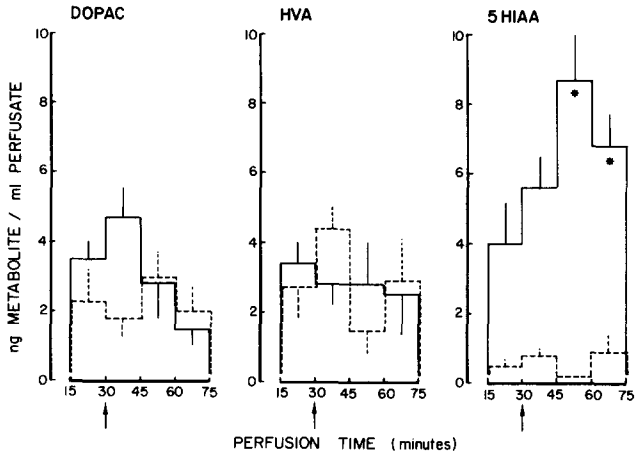

FIG. 1. Effects of L-tryptophan on DOPAC, HVA and 5HIAA concentrations in cerebroventricular perfusates of unanesthetized rats before and after treatment with 5,7-DHT. The lateral cerebroventricles of 4 rats were perfused with artificial cerebrospinal fluid for 75 min with sequential samples collected at 15 min intervals. Each animal was injected with L-tryptophan (100 mg/kg, IP) at 30 min as indicated by the arrows. Approximately 2 hr after the end of the perfusion, 5,7-DHT (200 μg/rat) was injected ICV. Ten days later the animals were again perfused and injected with L-tryptophan. Values represent the mean perfusate concentrations of DOPAC, HVA and 5HIAA before (—) and after (----) treatment with 5,7-DHT. Vertical lines represent 1 S.E.; *represents L-tryptophan-induced changes in metabolite concentrations ($p<0.05$).

in subsequent samples from each individual animal is generally quite consistent during the course of each experiment and is not altered by injection of saline or drug vehicle [11].

In animals treated with 6-OHDA, the basal efflux of DOPAC and HVA, but not of 5HIAA, was markedly reduced at 7 days and decreased even more at 14 days (Table 1). Confirming previous findings [11], haloperidol markedly increased the efflux of the DA metabolites, but was without effect on 5HIAA; in 6-OHDA-treated rats, the action of haloperidol was greatly attenuated. These results are consistent with previous reports in which 6-OHDA destroyed DA neurons, but not 5HT neurons [2, 4, 9]. Gross histological damage was not seen in brains of 6-OHDA-treated rats (see also [12]).

In animals treated with 5,7-DHT, the basal efflux of 5HIAA was reduced to approximately 10% of pretreatment values, while the efflux of DOPAC and HVA was unaltered (Fig. 1). In control animals, L-tryptophan increased the efflux of 5HIAA but not DA metabolites; the former effect was not seen in 5,7-DHT-treated rats. Moderate brain tissue damage was observed in 5,7-DHT-treated animals, especially in periventricular areas.

In summary, these results suggest that the metabolites of DA and 5HT appearing in cerebroventricular perfusates originate from DA and 5HT neurons in the brain, and indicate that this *in vivo* perfusion technique can be employed to monitor the activity of these neurons in conscious, unrestrained rats.

ACKNOWLEDGEMENTS

This work was supported by USPHS Grant NS15911. The authors wish to thank Dave LaClair for technical assistance and Diane Hummel for manuscript preparation.

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