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Behavioral Concomitants of Regional Changes in the Brain's Biogenic Amines After Apomorphine and Amphetamine

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SCHWARTING, R. K. W. AND J. P. HUSTON. *Behavioral concomitants of regional changes in the brain's biogenic amines after apomorphine and amphetamine.* PHARMACOL BIOCHEM BEHAV **41**(4) 675-682, 1992.—Behavioral and neurochemical changes were analyzed in rats after systemical injections of the dopamine receptor agonist apomorphine (0.5 mg/kg) or the indirect agonist amphetamine (1.0 mg/kg). As expected, amphetamine led to an increase in locomotion, whereas apomorphine resulted in decreases in locomotion, rearings, and grooming. The analysis of biogenic amines in tissue samples showed that amphetamine decreased 3,4-dihydroxy-phenylacetic acid (DOPAC) levels and DOPAC/dopamine ratios in the neostriatum, and resulted in a lower 5-hydroxyindole-3-acetic acid (5-HIAA)/5-hydroxytryptamine (5-HT) ratio in the ventral mesencephalon. Apomorphine decreased the dopamine metabolites [DOPAC, homovanillic acid (HVA), 3-methoxy-tyramine (3-MT)] and their respective metabolite/transmitter ratios and increased dopamine levels in the neostriatum. Similar decreases in dopamine metabolites or their ratios were found in the ventral mesencephalon, septum, and frontoparietal cortex but not the thalamus. In addition to its effects on dopamine, apomorphine decreased norepinephrine in the ventral neostriatum and 5-HT and 5-HIAA in the cortex. Correlations between behavioral activity and neurochemical metabolism (using the metabolite-transmitter ratios for the latter) revealed relationships between locomotion and serotonergic activity in the thalamus of animals treated with amphetamine. Evidence for a relationship between locomotion or rearings and dopaminergic activity was found in all six brain areas analyzed. Here, the pattern of correlation was dependent on the kind of treatment and the behavioral and neurochemical measures. These results support earlier findings on the neurochemical effects of apomorphine and amphetamine in the neostriatum and ventral mesencephalon, and add new evidence for an action on the septal area, thalamus, and frontoparietal cortex.

Dopamine Serotonin Norepinephrine Neostriatum Septum Cortex Thalamus
Ventral mesencephalon Open-field behavior Rat

THE indirect agonist amphetamine (AMPH) and the direct receptor agonist apomorphine (APO) have been widely used for the study of the neurotransmitter dopamine (DA), especially with respect to its role in behavior [for reviews, see (4,22)].

With respect to the anatomical sites of action of these drugs, the most attention has been given to areas of dense DAergic innervation, such as the neostriatum, nucleus accumbens, substantia nigra (SN), ventral tegmental area (VTA), or the prefrontal cortex. Compared to these, other ares of known DAergic input such as the septum, or areas with minimal DA input, such as the sensorimotor cortex or the thalamus, have been neglected. Since we found interaction of these DAergic drugs with experimental manipulations such as vibrissotomy or brain lesions [e.g., (29,30,41,45)], it was of interest to investigate the neurochemical changes induced by these drugs more closely and compare them with the concomitant changes in behavior. For this purpose, we applied our standard testing conditions to relate the present results with those of our previous work. These conditions comprise 1) our standard drug doses, 2) the interval between injection and behavioral testing, and 3) the kind of apparatus for behavioral testing. Thus, groups of rats received either 1.0 mg/kg AMPH or 0.5 mg/kg APO. Control animals were administered the vehicle. Behavioral testing in the open field was performed 10 min after drug injection. The neurochemical analysis was performed post mortem in brain samples. This technique was preferred to in vivo methods such as microdialysis [which is also established in our laboratory; (1)] due to the following reasons: 1) They are not suitable for the screening of multiple brain areas, 2) brain areas with very low extracellular transmitter levels are extremely difficult to analyze, and 3) treatments that decrease transmitter release (such as APO) can lead to undetectable low transmitter levels.

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Thus, in the present study brain samples from neostriatum, septum, sensorimotor cortex (frontoparietal), thalamus, and ventral mesencephalon were analyzed for levels of biogenic amines. The metabolite/transmitter ratios, as an index of transmitter metabolism, were used to correlate neurochemical and behavioral activity in individual animals.

METHOD

Subjects

Male Wistar rats, weighing 175–225 g at the beginning of the experiment, were housed in groups of five animals under standard laboratory conditions. They had free access to food and water, were handled for several days, and were maintained under a 12 L:12 D cycle with lights on at 0700 h.

Drugs and Behavioral Testing

Animals were assigned to three groups. The AMPH group (n = 16) received 1 mg/kg +−desoxynorephedrin-sulfate (Knoll AG); the APO group (n = 18) received 0.5 mg/kg APO (Woelm Pharma); and the VEHICLE group (n = 16) received the solvent (0.9% saline). The injection volume in every group was 2 ml/kg, and injections were given subcutaneously (in the neck). After injection, the animal was placed in a separate cage for 10 min, after which behavioral testing was performed.

For this purpose, the animal was placed into an open-field apparatus that consisted of a black wooden chamber (60 × 60 × 40 cm) with a black floor. Behavior was monitored by a video camera positioned above the center of the chamber (6). Behavioral recordings were performed under dim red light. The following behavioral parameters were recorded over a period of 5 min immediately after placing the rat into the open field: 1) locomotion, that is, distance traversed by the rat (expressed in m); 2) rearing, that is, the number of times the rat reared up on its hind legs; and 3) grooming, that is, the number of grooming bouts independent of their length.

Neurochemical Analysis

Immediately after behavioral testing, that is, about 15 min after drug administration, animals were lightly anesthetized with ether and decapitated. The brains were dissected out on ice and sliced by hand, using the shape of the ventral surface of the brain and blood vessels as landmarks. Six areas of the brain were dissected out: 1) a cortical sample, including parietal cortex (area 1, 2), frontal cortex (area 1, 3), forelimb, and hindlimb area [according to plates 11–20; (35)]; 2) septum (ventrally bordered by the edges of the lateral ventricles and caudally by the level of the anterior commissure, where it crosses the midline; 3) and 4) neostriatum (according to plates 11–20; ventrally bordered by the level of the anterior commissure), which was divided into a ventral and a dorsal half; 5) thalamus (according to plates 29–35; ventrally bordered by the dorsal zona incerta); and 6) ventral mesencephalon (according to plates 37–43; dorsally bordered by the level of the peripeduncular nucleus). Samples were taken unilaterally; the left or right side was chosen randomly from animal to animal.

Samples were handled and analyzed by means of high-performance liquid chromatography with electrochemical detection using methods described elsewhere (39,40). The following substances could be detected (in the order of chromatographic elution): NE, dihydroxybenzylamine (DHBA; internal standard), 3,4-dihydroxy-phenylacetic acid (DOPAC), DA, 5-hydroxyindole-3-acetic acid (5-HIAA), homovanillic acid (HVA), 3-methoxytyramine (3-MT), and 5-hydroxytryptamine (5-HT).

Statistical analysis. Results are expressed as means with standard errors of the mean. Behavioral data were compared between vehicle- and drug-treated animals by way of one-tailed t-tests (α = 0.05). Due to the multiple testing (VEHICLE vs. AMPH, VEHICLE vs. APO), the significance level was adjusted to $\alpha/2$ (0.025) according to the Bonferroni procedure (23). Concentrations of neurochemical substances or metabolite-transmitter (DOPAC/DA, HVA/DA, 3-MT/DA, 5-HIAA/5-HT) ratios were compared between vehicle- and drug-treated animals using one-way analysis of variance (ANOVA) and posthoc Scheffe's tests. Finally, metabolite/transmitter ratios and behavioral data were correlated individually using Spearman's rank correlation.

RESULTS

Neurochemical Measures

Biogenic amines in vehicle-injected animals (Table 1). Except for HVA in cortex, thalamus, and septum and 3-MT in cortex, thalamus, septum, and ventral mesencephalon, 5-HT, 5-HIAA, NE, DA, DOPAC, HVA, and 3-MT could be analyzed in all six brain areas. The highest levels of 5-HT, 5-HIAA, and NE were found in the ventral mesencephalon, whereas the lowest levels of 5-HT and NE were measured in the dorsal and ventral neostriatum and the lowest levels of 5-HIAA were found in the dorsal neostriatum and the cortex. DA and its metabolites were highest in the neostriatum and lowest in the cortex and thalamus. The 5-HIAA/5-HT ratios were highest in the neostriatum and thalamus and lowest in the cortex. The DOPAC/DA ratios, on the other hand, were highest in the cortex and lowest in neostriatum and septum. Comparison between dorsal and ventral neostriatum showed that 5-HT, 5-HIAA, NE, DA, and HVA levels were higher in the ventral part (two-tailed t-tests for dependent samples; p values between 0.047 and <0.00001). The HVA/DA ratio was higher in the ventral part (p = 0.002), whereas 5-HIAA/-5-HT was lower (p = 0.0005) in this sample.

Effects of AMPH. Levels of DOPAC were significantly reduced (p < 0.00001) in the ventral and dorsal neostriatum to about 70% of vehicle-injected animals (Table 2). Accordingly, the DOPAC/DA ratios (Table 3) in these structures were also reduced (p < 0.0001). Except for decreased 5-HIAA/5-HT ratios in the ventral mesencephalon (p = 0.013), there were no other significant changes after AMPH (p > 0.05).

Effects of APO. Levels of DA were increased both in the ventral (p = 0.0004) and dorsal (p = 0.016) neostriatum (Table 2), but remained unaffected in the other brain areas. DOPAC levels were reduced in the neostriatum (p < 0.00001), septum (p = 0.0008), and ventral mesencephalon (p = 0.0004). The respective DOPAC/DA ratios were reduced in the neostriatum, ventral mesencephalon, and cortex (p between 0.035 and <0.00001; Table 3). HVA levels were significantly lower in ventral and dorsal neostriatum (p = 0.011) and slightly lower in the ventral mesencephalon. HVA/DA ratios were lower in all three brain areas (p between 0.018 and 0.0002). 3-MT and 3-MT/DA ratios were lower both in dorsal and ventral neostriatum (p between 0.014 and 0.0005). The levels of NE were unaffected in cortex, mesencephalon, and thalamus, slightly but nonsignificantly (p > 0.05) re-

TABLE 1
NEUROCHEMICAL RESULTS IN VEHICLE-INJECTED ANIMALS

	Cor	Thal	Sep	Mes	NSv	NSd
Tissue Weights	29.82 ± 1.72	31.67 ± 1.07	4.85 ± 0.30	16.77 ± 1.07	12.14 ± 0.70	12.16 ± 1.07
Substances						
5-HT	354 ± 24	350 ± 23	375 ± 69	944 ± 38	292 ± 15	149 ± 8
5-HIAA	232 ± 13	514 ± 28	420 ± 36	950 ± 39	417 ± 21	259 ± 18
NE	156 ± 9	296 ± 18	386 ± 54	575 ± 16	100 ± 11	40 ± 8
DA	50 ± 7	78 ± 8	796 ± 67	767 ± 39	8,510 ± 249	7,739 ± 394
DOPAC	32 ± 4	23 ± 1	117 ± 17	196 ± 13	1,366 ± 57	1,273 ± 60
HVA	nd	nd	nd	164 ± 11	726 ± 75	566 ± 60
3-MT	nd	nd	nd	nd	388 ± 45	355 ± 38
Ratios						
5-HIAA/5-HT	0.672 ± .030	1.500 ± .069	0.723 ± .121	1.008 ± .020	1.449 ± .061	1.747 ± .076
DOPAC/DA	0.700 ± .072	0.326 ± .028	0.141 ± .022	0.252 ± .006	0.160 ± .004	0.166 ± .005
HVA/DA	nd	nd	nd	0.213 ± .007	0.085 ± .008	0.073 ± .006
3-MT/DA	nd	nd	nd	nd	0.045 ± .005	0.046 ± .004

Tissue weights are given as mg of wet weight (means ± SEM; $n = 16$). The concentrations of neurochemical substances are given in ng/g brain tissue (mean ± SEM). nd, not detected. Brain sites: Cor, parietal cortex; Thal, thalamus; Sep, septum; Mes, ventral mesencephalon; NSv, ventral neostriatum; NSd, dorsal neostriatum.

duced in septum and dorsal neostriatum, and significantly reduced in the ventral neostriatum ($p = 0.009$). 5-HT and 5-HIAA levels were unaffected in the neostriatum, septum, thalamus, and mesencephalon, but decreased in the cortex (p between 0.027 and 0.007). Their respective metabolite/transmitter ratios were not significantly different from those of vehicle-injected animals in any of the six brain areas.

Behavioral Measures

Compared to vehicle-injected animals (Table 4), locomotor activity (measured as distance traversed in meters) was increased after AMPH ($p = 0.005$) and decreased after APO ($p < 0.00001$). The number of rears was not significantly affected by AMPH and drastically reduced by APO ($p < 0.00001$). The frequency of grooming bouts was low and variable both in vehicle- and AMPH-treated rats. After APO, grooming behavior was no longer observed. These animals were engaged in intensive and continuous sniffing behavior of floor and wall of the open field; however, this behavior was not quantified.

Correlations Between Neurochemical and Behavioral Measures

Except for a positive correlation between thalamic 5-HIAA/5-HT ratios and locomotion in vehicle- and AMPH-treated animals, there were no substantial correlations between the serotonergic and behavioral measures (Table 5). DOPAC/DA ratios in the neostriatum were not correlated with behavior; however, in the ventral mesencephalon they were positively correlated with locomotion in vehicle- and APO-treated animals. Furthermore, in control animals locomotion was positively correlated with the cortical DOPAC/DA ratios and rears with the septal DOPAC/DA ratios. In AMPH-treated animals, the thalamic DOPAC/DA ratio was negatively correlated with locomotion and that in the septum with both behavioral measures. Finally, in vehicle-injected animals the 3-MT/DA ratio was negatively correlated with locomotion in both striatal subsamples.

DISCUSSION

Biogenic Amines in Vehicle-Injected Animals

The tissue levels of aminergic transmitters are well in accord with those reported by others (34,37,47). Thus, the levels of DA were high in the neostriatum, intermediate to low in the mesencephalon and septum, and very low in the frontoparietal cortex and thalamus. NE and 5-HT were more evenly distributed, with the highest concentrations in the mesencephalon and the lowest in the neostriatum. Using the metabolite/transmitter ratios as an index of transmitter metabolism indicated that the metabolism of 5-HT and DA is different in different brain areas. For example, the DOPAC/DA ratio was considerably higher in brain areas with low DA levels, such as the cortex, than with high DA levels, such as the neostriatum, supporting previous results (17). These different ratios may reflect differences in the rate of synthesis, basal release, reuptake, enzymatic degradation, or self-regulatory feedback mechanisms, which have been shown to differ between different systems of a given aminergic transmitter [e.g., (2,17,20, 43,49)]. Even within one structure, transmitter levels and metabolism may be heterogeneously distributed. Thus, within the neostriatum the dorsal part showed lower concentrations of all three transmitters than the ventral part, which in the case of NE and 5-HT supports earlier results (3,46,51). Furthermore, the HVA/DA and 5-HIAA/5-HT ratios indicated a higher turnover of DA in the ventral part and a higher turnover of 5-HT in the dorsal part, which is also in accord with previous results (3). In the case of DA, the differences between the dorsal and the ventral neostriatum may be related to different sources of input, as the dorsal part is mainly innervated by the SN whereas in the ventral part the DAergic innervation is provided mainly by the VTA [for review, see (5)].

Effects of AMPH

Behaviorally, it was found that 1 mg/kg AMPH led to increased levels of locomotion, which is typically observed with low doses such as were used here [for review, see (22)].

TABLE 2

NEUROCHEMICAL EFFECTS IN DRUG-TREATED ANIMALS

Substances	AMPH						APO					
	Cor	Thal	Sep	Mes	NSv	NSd	Cor	Thal	Sep	Mes	NSv	NSd
5-HT	90 ± 6	113 ± 8	93 ± 20	113 ± 6	104 ± 6	92 ± 7	77 ± 4*	109 ± 9	87 ± 12	105 ± 4	100 ± 6	96 ± 5
5-HIAA	87 ± 5	97 ± 4	114 ± 11	98 ± 4	98 ± 4	90 ± 5	78 ± 4†	98 ± 7	94 ± 6	107 ± 6	93 ± 5	86 ± 6
NE	114 ± 7	108 ± 7	111 ± 16	103 ± 3	112 ± 10	122 ± 20	100 ± 4	89 ± 6	73 ± 8	100 ± 2	59 ± 7†	52 ± 10
DA	116 ± 12	90 ± 6	98 ± 11	93 ± 5	111 ± 5	110 ± 4	112 ± 10	97 ± 8	85 ± 9	101 ± 5	124 ± 4‡	117 ± 3*
DOPAC	84 ± 12	78 ± 9	66 ± 19	87 ± 5	68 ± 3‡	68 ± 3‡	72 ± 9	96 ± 9	31 ± 10†	69 ± 3‡	55 ± 2‡	61 ± 2‡
HVA	nd	nd	nd	95 ± 6	89 ± 8	91 ± 8	nd	nd	nd	82 ± 4	64 ± 6*	62 ± 7*
3-MT	nd	nd	nd	nd	102 ± 11	122 ± 11	nd	nd	nd	nd	57 ± 7*	56 ± 6†

All values are expressed as percentage (mean ± SEM) of vehicle-treated animals. AMPH, 1.0 mg/kg amphetamine ($n = 16$); APO, 0.5 mg/kg apomorphine ($n = 18$). *$p < 0.05$; †$p < 0.01$; ‡$p < 0.001$: different from vehicle according to ANOVA and Scheffe's tests. nd, not detected. Abbreviations for brain sites according to Table 1.

TABLE 3

METABOLITE/TRANSMITTER RATIOS IN DRUG-TREATED ANIMALS

Ratio	AMPH						APO					
	Cor	Thal	Sep	Mes	NSv	NSd	Cor	Thal	Sep	Mes	NSv	NSd
5-HIAA/5-HT	98 ± 5	88 ± 5	89 ± 18	87 ± 2*	94 ± 3	101 ± 4	102 ± 6	93 ± 8	105 ± 16	101 ± 4	93 ± 4	90 ± 4
DOPAC/DA	73 ± 13	84 ± 12	65 ± 21	94 ± 2	62 ± 2†	61 ± 2†	62 ± 7	97 ± 13	66 ± 30	70 ± 3†	45 ± 1†	51 ± 2†
HVA/DA	nd	nd	nd	102 ± 4	84 ± 8	85 ± 8	nd	nd	nd	83 ± 5*	52 ± 5†	53 ± 5†
3-MT/DA	nd	nd	nd	nd	96 ± 11	113 ± 11	nd	nd	nd	nd	47 ± 4†	48 ± 4†

All values are expressed as percentage (mean ± SEM) of vehicle-injected animals. AMPH, 1.0 mg/kg amphetamine ($n = 16$); APO, 0.5 mg/kg apomorphine ($n = 18$). nd, not detected. *$p < 0.05$, †$p < 0.001$: different from vehicle according to ANOVA and Scheffe's test. Abbreviations of brain sites according to Table 1.

TABLE 4

BEHAVIOR IN THE OPEN-FIELD TEST

	Locomotion	Rearing	Grooming
Vehicle	16.98 ± 0.77	33.56 ± 2.60	.94 ± 0.32
AMPH (1 mg/kg)	20.47 ± 1.02*	37.56 ± 3.07	.50 ± 0.44
APO (0.5 mg/kg)	7.28 ± 0.94†	1.50 ± 0.53†	.00 ± 0.00*

Locomotion was measured as the distance traversed in meters. Rearing was counted as the number of times the rat reared on its hind legs; grooming was counted as the number of grooming bouts (means ± SEM). One-tailed t-tests with α-adjustment ($\alpha/2$): *$p < 0.005$, †$p < 0.0005$.

Neurochemical analysis revealed that AMPH affected DAergic measures in the neostriatum, where DOPAC and its metabolite/transmitter ratio was decreased. Furthermore, 5-HIAA/5-HT ratios were reduced in the ventral mesencephalon. The decrease of neostriatal DOPAC and the lack of change in HVA is well in accord with other results using similar or identical doses (11,12,42). With respect to the underlying mechanisms, it is assumed that AMPH has at least three modes of action on DAergic neurons: 1) increased release of DA from a cytoplasmic pool, 2) inhibition of reuptake, and 3) inhibition of MAO, one of its degrading enzymes (24). All these mechanisms can lead to decreased DOPAC levels, as this metabolite is formed mainly intraneuronally via MAO.

In contrast to the striatum, information about AMPH-induced effects on aminergic tissue levels in other brain areas is sparse. Segal and Kuczenski (42) found no effect of 1.75 mg/kg AMPH on cortical NE, DA, and its metabolites, which is confirmed here with a lower dose. Similar to the cortical sample, no significant effects were found on DAergic measures in thalamus, septum, and ventral mesencephalon in the present study. However, these results should not be interpreted in the sense that AMPH has no effect on DA in these brain areas as the time course of neurochemical change might be different here. Thus, 1 h after drug application a decrease of HVA was found in the SN (32) with 5 mg/kg, whereas a decrease of DOPAC was found in another study with 0.25–1.25 mg/kg (12). In a dialysis study, 0.67 mg/kg, in contrast to 2.2 or 6.7 mg/kg, only weakly increased DA release in the A10 area (19). These differences point out the importance of drug dosage and time of testing.

In addition to its effects on DA, AMPH influenced the metabolism of 5-HT, which was also indicated by previous studies (25,42). However, in contrast to these studies the changes did not occur in the neostriatum and cortex but in the ventral mesencephalon, which had not received much attention in this regard before. An interaction between AMPH and 5-HT has been suggested both by behavioral and neurochemical studies [e.g., (8,36)]. The present experiment supports such an interaction and points at a role of serotonergic systems in the ventral mesencephalon.

Effects of APO

Behaviorally, the DA receptor agonist APO in a dose of 0.5 mg/kg reduced locomotor activity (i.e., the distance traversed), rearings, and grooming. Nevertheless, these animals were not inactive as they continuously sniffed the wall and floor of the environment. Regarding locomotor activity, studies using identical doses have reported contradictory results such as reduction (15,31), no change, or even increases (13, 21,28). These contradictory results may be due to different definitions of locomotor activity and the methods to measure it. Thus, some measures are confounded by motor activity, which is increased after APO but does not necessarily require locomotion, that is, the traversion of distance. Accordingly, in another study (31) that reported a reduction of locomotor activity this behavior was also measured as distance traversed.

Neurochemically, APO produced well-known decreases of mesencephalic and neostriatal DA metabolites and metabolite/transmitter ratios, including striatal 3-MT levels [e.g., (11, 32,48,50,53)], and extended these findings for the septum and frontoparietal cortex. This latter finding supports previous results indicating that the sensorimotor cortex also receives a DAergic innervation (18). Furthermore, increases of neostriatal DA levels were found, which have also been reported by other authors (33,53). APO is thought to act on DAergic neurons via negative feedback mechanisms, leading to decreased transmitter synthesis, firing rate, and release. These mechanisms can lead to decreased metabolite levels as they decrease the availability of DA for enzymatic degradation. However, the increases in neostriatal DA levels appear surprising as a decrease in synthesis should be followed, if at all, by decreased transmitter levels. If one assumes that the inhibition of synthesis and that of release follow a different time course or reach a different degree, the discrepancy becomes explainable. Thus, if the inhibition of release occurs earlier or is stronger than that of synthesis DA levels might increase temporarily inside the cell, leading to a small increase of tissue levels of DA. A similar conclusion was drawn by Boyar and Altar (7), who found an increased neostriatal level of DA after a selective D_2 agonist and argued that this selective agonist might have had a stronger inhibitory effect on DA release and metabolism than on synthesis.

In addition to its effect on DA, APO influenced the metabolism of 5-HT as the levels of 5-HT and 5-HIAA were reduced in the frontoparietal cortex. Previous results also indicated an influence of APO on serotonergic systems. However, in these experiments increased levels of 5-HT or 5-HIAA were found in total brain, neostriatum, or frontal cortex using similar or higher doses (16,25,38). It was suggested that such serotonergic effects of APO are mediated indirectly, that is, via DA and an input from the SN to the nucleus raphe dorsalis, which comprises the major origin of telencephalic 5-HT systems (26,27). Why APO decreased 5-HT and 5-HIAA levels in the present study but increased them in previous experiments remains unclear. Apart from differences in sample outline, a shorter interval between drug injection and neurochemical analysis was used here (15 vs. 30 min or longer). Thus, it could

TABLE 5
CORRELATIONS BETWEEN NEUROCHEMICAL AND BEHAVIORAL MEASURES

		5-HIAA/5-HT		DOPAC/DA		HVA/DA		3-MT/DA	
		Locomotion	Rearing	Locomotion	Rearing	Locomotion	Rearing	Locomotion	Rearing
Ventral Neostriatum	Vehicle	−0.05	0.18	−0.14	0.10	−0.23	0.20	−0.48*	−0.18
	AMPH	0.16	−0.05	−0.06	−0.28	−0.16	−0.02	−0.08	0.15
	APO	0.04		−0.14		0.12		0.01	
Dorsal Neostriatum	Vehicle	−0.14	−0.39	−0.09	−0.06	−0.23	0.02	−0.53*	−0.33
	AMPH	−0.18	−0.38	−0.06	−0.19	−0.30	−0.09	−0.28	−0.04
	APO	0.21		0.03		−0.05		0.21	
Ventral Mesencephalon	Vehicle	−0.25	0.38	0.42*	0.38	0.34	0.32		
	AMPH	0.13	0.05	−0.04	0.03	−0.23	−0.08		
	APO	−0.11		0.41*		0.39			
Frontoparietal Cortex	Vehicle	0.36	0.23	0.46*	0.03				
	AMPH	−0.19	0.01	−0.01	0.16				
	APO	−0.37		0.19					
Septum	Vehicle	−0.20	−0.11	0.25	0.51*				
	AMPH	0.29	0.22	−0.51*	−0.58*				
	APO	0.24		0.19					
Thalamus	Vehicle	0.43*	0.25	−0.41	0.01				
	AMPH	0.52*	0.26	−0.47*	−0.34				
	APO	−0.39		0.15					

Correlations between locomotion or rearing and metabolite/transmitter ratios in rats injeted with either vehicle, amphetamine (AMPH; 1 mg/kg), or apomorphine (APO; 0.5 mg/kg). The correlational analysis was performed using Spearman's rank correlation coefficient followed by the rank correlation test; *$p < 0.05$, not corrected for multiple testing.

be assumed that the effect of APO on 5-HT is time dependent, that is, initially leading to decreased levels followed by increased levels thereafter.

Correlations Between Behavior and Transmitter Metabolism

The metabolite/transmitter ratios were used to correlate neurochemical and behavioral activity. This analysis revealed several moderate positive and negative correlations (no higher than 0.577). Due to the number of tests, a single result should not be overinterpreted; instead, the pattern of correlation might provide useful information. In this sense, some results are interesting to note.

In the neostriatum, there were no substantial correlations between neurochemical and behavioral measures except for the 3-MT/DA ratios in the dorsal and ventral neostriatum, which were negatively correlated with the level of locomotor activity in undrugged animals. If one assumes that 3-MT, which is formed from released DA, is closely related to DAergic activity [for review, see (52)] and that a higher ratio reflects higher activity, then it follows from the present results that a higher level of locomotor activity is accompanied by a lower level of DAergic activity in the neostriatum. This result is surprising as it is commonly accepted that a high level of behavioral activity is accompanied by a high level of DAergic activity [for review, see (4)]. However, it should be considered that various factors such as release, reuptake, and enzymatic degradation contribute to metabolite levels and thus to metabolite/transmitter ratios [for discussion, see (9)]. Therefore, these ratios should be taken as general indicators of transmitter metabolism and should not by themselves be attributed to one neuronal mechanism. In this sense, it remains to be demonstrated whether a low 3-MT/DA ratio reflects a low level of DA release in an undrugged animal.

The DOPAC/DA ratios were correlated with behavioral measures in ventral mesencephalon, frontoparietal cortex, septum, and thalamus. In vehicle-injected animals, this ratio was positively correlated with locomotor activity in mesencephalon and cortex and with the number of rears in the septum. Thus, in contrast to the neostriatum, the hypothesis of a positive relationship between behavioral (locomotion, rearing) and DAergic activity may hold for these brain areas. Furthermore, this assumption is supported by the results from AMPH-treated rats. Here, the DOPAC/DA ratios were negatively related to locomotion and rears in the septum and to rears in the thalamus. It might appear paradoxical to interpret these negative correlations after AMPH as supportive for a positive relationship between DAergic and behavioral activity. However, it should be kept in mind that the relevant mechanism of AMPH, that is, the increased availability of extracellular DA, is accompanied by reductions in tissue levels of DOPAC and thus DOPAC/DA ratios. Although such reductions were not statistically significant in the thalamus and septum, a tendency could be observed in both structures (Tables 2, 3). Thus, it can be hypothesized that the release of DA in parietal cortex and septum is positively related to mechanisms involved in locomotion and rearings. Again, this conclusion is limited by restrictions regarding the interpretation of metabolite/transmitter levels.

Finally, analysis of serotonergic activity indicated a relationship between this transmitter and behavior, however, only in the thalamus, where a positive correlation was found between 5-HIAA/5-HT and locomotor activity in undrugged and AMPH-treated animals. Knowledge about the role of thalamic 5-HT is sparse. Its innervation is densest in areas

such as the midline nuclei and the lateral geniculate bodies (10,44). Functionally, it is assumed that 5-HT may play a modulatory role here involving visual aspects, nociception, and an interaction with co-called limbic brain areas (10). Furthermore, 5-HT has repeatedly been reported to play a role in locomotor activity [for review, see (14)]. However, to our knowledge the thalamus has not been considered in this specific context. The present results support the relationship between 5-HT and locomotion and provide new evidence for a role of thalamic 5-HT.

Taken together, the present results support previous data regarding behavioral and neurochemical effects of AMPH and APO. Furthermore, they provide new evidence for influences on biogenic amines in septum, thalamus, and sensorimotor cortex and support the involvement of these transmitters in open-field behavior. The correlations between behavioral activity and the metabolism of DA and 5-HT in cortex, septum, and thalamus warrants further research on biogenic amines in these brain areas.

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