Effects of Neurotransmitters or Drugs on the *in Vivo* Release of Dopamine and Its Metabolites

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Abstract—The effects of neurotransmitters or drugs on the release of endogenous dopamine (DA) and extracellular levels of its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), were examined *in vivo* by intracerebral dialysis. A dialysis tube was implanted stereotactically through bilateral caudate nuclei of rats and perfused with the Ringer solution. Amounts of DA, DOPAC and HVA in the perfusates were measured by high performance liquid chromatography (HPLC) with electrochemical detection. The basal level of DA was 2.76 ± 0.64 pg/min, whereas the levels of DOPAC and HVA were 218.7 ± 20.7 and 142.4 ± 10.6 pg/min, respectively. Apomorphine (4 mg/kg, *i.v.*) reduced the efflux of DA and its metabolites. Haloperidol (0.4 mg/kg, *i.v.*) did not change DA release and produced only a minor increase of its metabolites. This increase of metabolites was inhibited by pargyline. Met-enkephalin (10^{-4} M), substance P (10^{-4} M) and acetylcholine chloride (10^{-4} M) added to the perfusing medium increased the release of DA. Met-enkephalin also increased the release of DOPAC. γ -Amino-n-butyric acid (GABA, 10^{-4} M) reduced the release of DOPAC and HVA when added to the perfusing medium. Thyrotropin releasing hormone (TRH, 5 mg/kg, *i.v.*) increased the release of HVA. These findings indicated that different mechanisms mediated effects of neurotransmitters or drugs on the release and metabolism of DA in the rat striatum.

Most of the *in vivo* and *in vitro* studies on the release of DA from nerve terminals have been done on the nigrostriatal DA system. This is due to a high density of DA terminals in the striatum and to the well-known anatomical organization of this pathway. *In vitro* studies have generally been carried out on rat striatal slices or synaptosomes preloaded with radiolabelled DA or DA precursors (1–3). *In vivo* studies allow the investigation of the interneuronal regulations of the activity of the nigrostriatal DA system in physiological states. Several attempts have been made to develop methods suitable for perfusing the extracellular environment. Gaddum (4) created the push-pull cannula, and this technique has been extensively applied to the study of transmitter physiology (5, 6). Delgado et al. (7) attempted a new

technique by implanting dialysis membranes in the form of a small dialysis bag. Ungerstedt et al. (8) recently developed an intracerebral dialysis technique that involved the continual perfusion of the rat striatum using an implanted loop of small-calibered dialysis tubing. Both techniques met with obvious difficulties related to the small amount of endogenous chemicals. However, development of highly sensitive HPLC techniques has made possible the extensive application of the dialysis technique to studies of brain chemistry (9, 10). The authors carried out an intracerebral dialysis of the striatum of rats similar to that reported by Ungerstedt et al. (8). The present study was to examine the effects of various transmitters or drugs on the release of endogenous DA from the rat striatum *in vivo*.

Materials and Methods

Intracerebral dialysis procedure: Male Wistar strain rats weighing 180–200 g were anaesthetized with pentobarbital and placed on a stereotaxic apparatus. One hole was drilled on each side of the temporal bone at the level of the head of the caudate nucleus (coordinates: A, 7.4; V, 5.5, according to the atlas of Köning and Klippel (11)). Through these holes, a straight dialysis tube (Hollow fibres, Amicon), with an outer diameter of 0.5 mm and a molecular weight cut-off of 50,000, was inserted transversely. Specific areas on the surface of the dialysis tube had previously been coated with epoxy to exclude dialysis in all areas except the striata. Six days after implantation of the dialysis tube into the brain, one end of the tubing was connected to a constant flow perfusion pump, and the Ringer solution was pumped into the tube. The Ringer solution contained 147 mmol Na⁺, 2.3 mmol Ca²⁺, 4 mmol K⁺ and 155.6 mmol Cl⁻ (pH 6.0). The perfusion speed was kept at 7 μ l/min. In accordance with the principle of dialysis,

molecules small enough to pass through the membrane of the tubing will pass in the direction of the lowest concentration of that particular molecule. Every 10 to 30 min, the Ringer solution was collected from the other end of the dialysis tube, and an aliquot of each fraction was directly injected into an HPLC apparatus. Figure 1 showed schematically the position of the dialysis tube in the caudate.

Determination of DA, DOPAC and HVA:

A Toyo Soda liquid chromatograph (Model 803 D) was used in conjugation with electrochemical detection. DA and its metabolites were separated by reverse phase chromatograph with octadecyl silica particles of 5 μ m using a mobile phase of 0.1 M citrate buffer, pH 5.2, containing 0.1 mM EDTA-Na₂ and 2.5% CH₃CN. Analysis was performed at a flow rate of 1 ml/min. Injection volume was 15 μ l.

Drugs: The following drugs were used: Haloperidol (Yoshitomi), apomorphine and pargyline (Sigma Chemicals), TRH (Takeda), substance P and met-enkephalin (Protein Research Foundation), GABA (Wako Pure

Fig. 1. Schematic drawing of the trans-striatal dialysis technique. The drawing shows a frontal section of the rat brain and the transversally inserted dialysis tube. Note that the tube is covered with epoxy glue in its extrastriatal portion.

Chemicals) and acetylcholine chloride (Daiichi). Haloperidol, apomorphine and TRH were administered intravenously dissolved in saline. Pargyline dissolved in saline was administered intraperitoneally. Substance P, GABA, met-enkephalin and acetylcholine chloride were added to the Ringer solution.

Results

Chromatogram of samples of dialysate:

Figure 2 showed a chromatogram of a sample of striatal perfusates taken under basal conditions. At the lowest sensitivity, DOPAC and HVA could be detected. At the higher sensitivity, DA and HVA were detected.

Fig. 2. Chromatogram of 15 μ l of dialysate. The upper part is the tracing obtained at the highest sensitivity on the two-pen recorder showing the peaks of DA and HVA. The lower part is the tracing obtained at the lowest sensitivity showing the peaks of DOPAC and HVA.

Time course of the basal release of DA and metabolites: Figure 3 shows the time course over 2 hr of the basal release of DA, DOPAC and HVA. This time course was characterized by an initial phase, the first 1 hr during which DA release decreased, while DOPAC and HVA increased. In the view of this observation that the release of DA, DOPAC and HVA reached a plateau after 1 hr from the start of the dialysis, pharmacological treatments were applied after this interval.

Basal release of DA and its metabolites in striatal perfusates: Table 1 showed the basal release of DA and its metabolites in striatal perfusates prior to drug administration. Basal levels in the samples were determined to be : DA, 2.76 ± 0.64 pg/min (N=12); DOPAC, 218.7 ± 20.7 pg/min (N=6); and HVA, 142.4 ± 10.6 pg/min (N=6). It was notable that the basal release of DOPAC was 79 times greater than that of DA.

Effects of neurotransmitters or drugs on the release of DA and its metabolites in striatal perfusates: Apomorphine (4 mg/kg, i.v.) caused a marked decrease in DA in striatal perfusates over the 70 min period following the drug administration. Both

Fig. 3. Time course of the basal release of DA, DOPAC and HVA. Values are means $\pm$ S.E.M. of six experiments.

Table 1. Dopamine and its metabolites levels (basal) in striatal perfusates prior to drug administration

Compound		Basal levels (pg/min)
DA	(N=12)	2.76± 0.64
DOPAC	(N=6)	218.7 ±20.7
HVA	(N=6)	142.4 ±10.6

Each value is a mean±S.E.M. Perfusion rate is 7 µl/min.

Fig. 4. Effect of apomorphine (4 mg/kg, i.v.) on the levels of DA, DOPAC and HVA in striatal perfusates. Results are expressed as a percentage of basal values and are means±S.E.M. of four experiments. *P<0.05, **P<0.01, with respect to basal values.

DOPAC and HVA, together with DA, decreased (Fig. 4). Haloperidol (0.4 mg/kg, i.v.) was ineffective in increasing the release of DA when there were increases of DOPAC and HVA (Fig. 5). Administration of pargyline (70 mg/kg, i.p.) 1 hr after haloperidol (0.4 mg/kg, i.v.) resulted in a minor increase of DA release. On the other hand pargyline not only prevented the increases of DOPAC and HVA produced by haloperidol, but actually decreased them (Fig. 6). Met-enkephalin (10⁻⁴ M) produced an increase of DA release when added to the Ringer solution. DOPAC also increased together with DA

Fig. 5. Effect of haloperidol (0.4 mg/kg, i.v.) on the levels of DA, DOPAC and HVA in striatal perfusates. Results are expressed as a percentage of basal values and are means±S.E.M. of four experiments. *P<0.05, with respect to basal values.

(Fig. 7). Substance P (10⁻⁴ M) increased the release of DA when added to the Ringer solution, while DOPAC and HVA remained unchanged (Fig. 8). GABA (10⁻⁴ M) was without effect on the release of DA when added to the Ringer solution, but it reduced the release of DOPAC and HVA (Fig. 9). Acetylcholine chloride (10⁻⁴ M) enhanced the release of DA when added to the Ringer solution. The DA release was increased by two times with respect to the basal values (Fig. 10). There was no change in the release

Fig. 6. Effect of pargyline (70 mg/kg, i.p.) on the release of DA, DOPAC and HVA in rats pretreated with haloperidol (0.4 mg/kg, i.v.) 1 hr in advance. Results are expressed as a percentage of basal values and are means $\pm$ S.E.M. of three experiments. * $P < 0.05$, ** $P < 0.01$, with respect to basal values.

Fig. 7. Effect of perfusion with the Ringer solution containing 10^{-4} M met-enkephalin on the levels of DA, DOPAC and HVA. Results are expressed as a percentage of basal values and are means $\pm$ S.E.M. of four experiments. * $P < 0.05$, with respect to basal values.

Fig. 8. Effect of perfusion with the Ringer solution containing 10^{-4} M substance P on the levels of DA, DOPAC and HVA. Results are expressed as a percentage of basal values and are means $\pm$ S.E.M. of four experiments. * $P < 0.05$, with respect to basal values.

Fig. 9. Effect of perfusion with the Ringer solution containing 10^{-4} M GABA on the levels of DA, DOPAC and HAV. Results are expressed as a percentage of basal values and are means $\pm$ S.E.M. of four experiments. * $P < 0.05$, with respect to basal values.

Fig. 10. Effect of perfusion with the Ringer solution containing 10^{-4} M acetylcholine chloride on the levels of DA, DOPAC and HVA. Results are expressed as a percentage of basal values and are means $\pm$ S.E.M. of four experiments. * $P < 0.05$, ** $P < 0.01$, with respect to basal values.

Fig. 11. Effect of TRH (5 mg/kg, i.v.) on the levels of DA, DOPAC and HVA in striatal perfusates. Results are expressed as a percentage of basal values and are means $\pm$ S.E.M. of four experiments. * $P < 0.05$, with respect to basal values.

of DA and DOPAC following administration of TRH (5 mg/kg, i.v.), whereas only the release of HVA was increased (Fig. 11).

Discussion

The present study demonstrated that it was possible to measure the release of endogenous DA as well as extracellular levels of its metabolites in the rat striatum *in vivo* using intracerebral dialysis and HPLC combined with electrochemical detection. Such a method was based on the principle of dialysis. Through this principle, low molecular weight substances present outside the dialysis tube, cross, by way of simple diffusion along a concentration gradient, the dialytic membrane, enter the tube and are thus recovered in the Ringer solution flowing inside it. The basal extracellular levels of DOPAC and HVA were 52–79 times greater than DA in the rat striatum. The estimated levels of DA in this study (about 2.76 pg/min) corresponded well with those estimated by Ungerstedt (12) and Imperato and Di Chiara (13) (about 2.8 pg/min). Nevertheless, the values of the basal levels of DOPAC and HVA obtained by us were about 35% lower than those reported by Ungerstedt (12) and Zetterström and Ungerstedt (14). The reason for this discrepancy is unclear, but it might be related to the fact that different conditions have been used. Ungerstedt (12) and Zetterström and Ungerstedt (14) used a lower perfusion speed at the rate of 2 μ l/min, whereas the rate of 7 μ l/min was used in the present study. The low recovery of the acidic metabolites in the perfusates was probably due to the high perfusion speed. Apomorphine (4 mg/kg, i.v.), a DA agonist, caused a marked decrease of DA and its metabolites in the rat striatum. These findings were in agreement with *in vitro* studies on rat striatal slices (15–17). The decrease in striatal DA and its metabolites following apomorphine administration is mostly due to presynaptic control of DA synthesis. Further, postsynaptically mediated decrease of DA release, probably caused by inhibition of neuronal feedback loops, may decrease the release by inhibiting nerve impulse flow (14, 18, 19). Haloperidol (0.4 mg/min, i.v.), a DA antagonist, stimulated increases of

DOPAC and HVA, while the release of DA remained unchanged. Drugs which block central DA receptors (neuroleptics) are known to stimulate the synthesis and the metabolism of DA and the firing activity of DA neurons (20–22). Such effects have been taken as evidence for the existence of feedback or autoregulatory mechanisms in the dopaminergic system. However, an important difference between the effect of haloperidol on the release of DA and that on the metabolism of DA was observed. The discrepancy in the release of DA and its metabolites after haloperidol administration calls for a different mechanism for the two effects. A working hypothesis which explains the present data postulates that the neuroleptics-induced stimulation of DOPAC and HVA release, which represents their stimulatory action on DA synthesis, is mediated by blockade of terminal DA autoreceptors (22, 23). In contrast, the stimulatory action of neuroleptics on DA release would result from a stimulation of DA firing secondary to blockade of postsynaptic DA receptors (21, 24). In the light of this hypothesis, haloperidol at a dosage of 0.4 mg/kg appeared to be less potent in blocking postsynaptic DA receptors than blocking DA autoreceptors. Jovoy et al. (25) observed a rapid accumulation of DA in the striatum of rats after administration of monoamine oxidase (MAO) inhibitors. In agreement with the consequent increase of the intraneuronal concentration of DA, MAO inhibitors increase DA release (13). Administration of pargyline, an inhibitor of MAO, to haloperidol-treated rats resulted in a minor increase of DA release. These findings indicated that haloperidol treatment did not affect intraneuronal MAO activity. As expected, DOPAC and HVA release markedly decreased after pargyline. It is well established that the striatum contains several neurotransmitters, and these transmitters modify the release of DA. Met-enkephalin (10^{-4} M) produced an increase of DA when added to the Ringer solution. In addition, increased release of DOPAC was observed. The presence of opiate binding sites in the striatum, one third of which appear to be localized on the terminals of dopaminergic neurons of the

nigrostriatal pathway, has been documented (26), and activation of opiate receptors results in an increase in striatal DA synthesis and turnover (27, 28). However, the previously reported changes in DA metabolism have been postulated by some authors (29) to reflect intraneuronal events not associated with DA release from neurons, and the answer to the question of whether the stimulation of opiate receptors inhibits or facilitates the release of DA remains clouded in controversy. Species dependent differences in opiate effects may account for some of the disparate findings. However, the conflicting results in previous studies can not be explained solely on the basis of species or genetic differences. In fact, no effect (30, 31), increase (32, 33), and decrease (34) in the release of DA in the striatum of the rat in response to the administration of morphine or enkephalins have been reported. Most of the in vivo studies have indicated that morphine and enkephalins seem to have a facilitatory effect on the release of DA in the striatum, although the nature of this effect remains obscure (35). We concluded from the present experiments that administration of met-enkephalin resulted in increased DA release in the rat striatum in vivo. Substance P (10^{-4} M) increased the release of DA when added to the Ringer solution. The data indicated that substance P exerted a facilitatory influence on the nigrostriatal dopaminergic system. GABA (10^{-4} M) decreased the release of DOPAC and HVA when added to the Ringer solution. The release of DA tended to increase during the first 1 hr after application of GABA, although the difference between basal levels of DA and GABA-induced release of DA was not significant. Recently, there are some evidences that GABA alters the MAO-A catalytic activity qualitatively (36). The initial increase in striatal DA following GABA application is attributed to its inhibitory effect on MAO activity. Acetylcholine chloride (10^{-4} M) enhanced the release of DA when added to the Ringer solution. Several experiments suggested that centrally acting cholinomimetic agents increased, in relatively low dosage, turnover of DA in the brain (37–39). Giorgiueff et al. (37) speculated that the

effects of cholinomimetic drugs were probably due to stimulation of muscarinic receptors since effects on DA release were prevented by centrally active anticholinergic agents like atropine. TRH (5 mg/kg, i.v.) caused an increase of HVA, whereas DA and DOPAC remained unchanged. The evidence suggested that TRH did not have a direct action on the nigrostriatal dopaminergic system. In conclusion, this study indicated that several interneuronal processes can be involved in the regulation of DA release from DA terminals. Furthermore, the results emphasize the usefulness of the brain dialysis technique for monitoring changes in neurotransmitter release and metabolism.

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