DOPAMINE RELEASE AND METABOLISM IN AWAKE RATS AFTER SYSTEMIC NEUROLEPTICS AS STUDIED BY TRANS-STRIATAL DIALYSIS¹

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Abstract

The method of trans-striatal dialysis has been applied here to the study of the release and metabolism of dopamine (DA) in the awake rat. DA and its acidic metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), present in the dialysates were separated by high performance liquid chromatography on reverse phase columns and estimated by electrochemical detection. In the awake rat, DA, DOPAC, and HVA could be recovered and quantitated in the dialysates for at least 4 days from the time of implantation of the dialysis tube. At a constant 2- μ l/min flow of Ringer in the dialysis tube, the output of the substances recovered in 20-min samples 24 hr after the implantation was as follows: DA, 0.318 ± 0.035 ; DOPAC, 41.3 ± 4.84 ; HVA, 32.98 ± 3.79 (mean picomoles $\pm$ SEM of six 40- μ l samples). The output of DA, DOPAC, and HVA decreased slowly so that 4 days after the implantation the output of DA was reduced by about 35% in respect to the 24-hr values. After a 24-hr recovery, drugs were administered and their effect on DA release and metabolism was investigated. Drugs of different chemical structure and spectrum, but having in common the property of blocking DA receptors and being effective neuroleptics such as haloperidol, sulpiride, and flupentixol, stimulated DA release and DOPAC and HVA output. Threshold doses for this effect were very low, being 0.012 mg/kg, s.c., for haloperidol, 2.5 mg/kg, s.c., for (-)-sulpiride, and 0.025 mg/kg, s.c., for cis-flupentixol. This effect was stereospecific as the (+) form of sulpiride and the trans- form of flupentixol were at least 10 to 100 times less potent than their enantiomer. The stimulation of DA release was shorter-lasting than the stimulation of DA metabolism and sedation or catalepsy. Moreover, whereas DA release did not increase by more than 100% over basal values, DOPAC and HVA increased by more than 3 times after maximally effective doses of neuroleptics. γ -Butyrolactone (200 mg/kg, i.p.) reversed haloperidol (0.1 mg/kg, s.c.), and sulpiride (20 mg/kg, s.c.) induced stimulation of DA release while it potentiated the stimulation of DOPAC and HVA output. These data indicate that stimulation of DA release by neuroleptics is strictly dependent upon stimulation of DA firing and that different mechanisms underline their effects on DA release and on DA metabolism.

Drugs which block central dopamine (DA) receptors (neuroleptics) are known to stimulate the synthesis and the metabolism of DA (Carlsson and Lindqvist, 1963; Roos, 1965; Sharman, 1966; Javoy et al., 1970; Andén et al., 1971; Andén, 1972; Carlsson, 1975; Scatton et al., 1975) and the firing activity of DA neurons (Bunney et al., 1973). Such effects have been taken as evidence for the existence of feedback or autoregulatory mechanisms in the dopaminergic system (Carlsson and Lindqvist, 1963; Bunney et al., 1973; Carlsson, 1975; Groves et al., 1975; Bunney and Aghajanian, 1976). According to this hypothesis the functional activity of the DA neurons is negatively

regulated by the amount of DA released from DA terminals or dendrites (Bjorklund and Lindvall, 1975; Geffen et al., 1976; Korf et al., 1976; Nieoullon et al., 1977) onto DA receptors located either postsynaptically in the caudate (Carlsson and Lindqvist, 1963; Bunney and Aghajanian, 1976) or on nondopaminergic terminals of the substantia nigra (Phillipson and Horn, 1976; Spano et al., 1976; Gale et al., 1977; Spano et al., 1977), or on the DA neurons themselves, either in the caudate (terminal autoreceptors) (Carlsson, 1975), or in the substantia nigra (nigral autoreceptors) (Aghajanian and Bunney, 1977). In spite of the large number of studies performed, the evidence for such mechanisms is derived from rather indirect indices of dopaminergic functional activity such as the synthesis and metabolism of DA *in vivo* (Carlsson and Lindqvist, 1963; Kehr et al., 1972; Di Chiara et al., 1977), the *in vivo* activation of tyrosine hydroxylase (Zivkovic and Guidotti, 1974; Zivkovic et al., 1975), the firing of DA neurons (Groves et al., 1975; Bunney and Aghajanian, 1976), and the behavior (Carlsson, 1975; Di Chiara et al., 1976). Indeed, the critical and essential step in this autoregulatory process, i.e., the release of DA, has been the subject of a rather limited number of studies, and often the results obtained are not unequivocal. *In vitro* studies, measuring DA release from synaptosomes or brain slices (Chéramy et al.,

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1970; Farnebo and Hamberger, 1971; Seeman and Lee, 1975; Westfall et al., 1976; Starke et al., 1978; Raiteri et al., 1979; Reimann et al., 1979; Arbilla and Langer, 1981; Kamal et al., 1981) have provided conflicting evidence and certainly are inadequate to study those regulatory effects which require the integrity of the DA neurons or of the neuronal circuits involving the caudate and the substantia nigra. *In vivo* studies all suffer the drawback of having been performed in anesthetized or encephale-isolé preparations (Stadler et al., 1975; Nieoullon et al., 1977, 1979). It is well known that anesthesia drastically affects the effect of neuroleptics on the synthesis and metabolism of DA as well as on the firing of DA neurons (Mereu et al., 1983). Moreover, in most studies performed *in vivo* on the effects of neuroleptics on DA release, the drugs have been administered locally in the brain rather than systemically (Nieoullon et al., 1977, 1979). Finally, most studies performed *in vivo* have measured the release of newly synthesized DA from precursor prelabeling rather than the release of endogenous DA (Nieoullon et al., 1977, 1979). Such ways of estimating DA release do not necessarily reflect the actual amount of DA released from the terminal since not only newly synthesized but also stored DA might contribute to released DA.

From these considerations it will appear that there is still the necessity of a study of the effects of systemically administered neuroleptics on the *in vivo* release of endogenous DA in awake animals.

Recently, we have described and validated the technique of trans-striatal dialysis coupled to high performance liquid chromatography (HPLC) as a method to study the *in vivo* release and metabolism of endogenous DA in the rat striatum (Imperato and Di Chiara, 1984). The *in vivo* DA release thus studied has the characteristics of an exocytotic release dependent upon depolarization of the terminals as it is strongly calcium dependent, is stimulated by 30 mM KCl and by electrical stimulation of the medial forebrain bundle, and is drastically reduced by administration of γ -butyrolactone, an agent known to block the firing of DA neurons. Drugs known to interfere with the synthesis, release, metabolism, or compartmentation of DA produce the expected changes in the release of DA and in the output of its metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) (Imperato and Di Chiara, 1984).

A drawback of the above technique, however, is the fact that DA release and metabolism are estimated in rats anesthetized with halothane. In order to avoid the artifacts of anesthesia and to correlate the *in vivo* DA release with behavior, we have modified the trans-striatal dialysis technique to apply it to awake rats.

In the present paper we describe the technique used, and we report the changes in the *in vivo* release and metabolism of DA produced by neuroleptics.

Materials and Methods

Male Sprague-Dawley rats were anesthetized with a 1.5 to 2.0% halothane-oxygen mixture and placed on a stereotaxic apparatus (David Kopf Instruments); then two trephine holes were made in the cranium at the level of the head of the caudate nucleus (coordinates A 7.4, V 5.5 from temporal bone) according to the atlas of König and Klippel (1970). Through these holes a thin dialysis tube (0.2 mm outer diameter) (Amicon Vitafiber, type X acrylic copolymer with a 50,000 molecular weight cutoff) was inserted transversally through both nuclei. The tube was mounted on a stainless steel wire and covered with Super-Epoxy glue through its whole extent except for two zones, one on each side, 1 mm wide, correspondent to the caudate of each side. The procedure used to insert the dialysis tube was essentially the same already described except that, in the present case, the entire length of the dialysis tube (5.7 cm) was used. In this way, after the stainless steel was extracted from the dialysis tube, its extracranial portions were fastened by dental cement (Sevriton) to the parietal bone. Each end of the dialysis tube was connected to a 22 gauge needle inserted into an Eppendorf tip and secured to it with Sevriton. The assembly composed

by the dialysis tube, the needle, and the Eppendorf tip was then secured in a vertical position to the parietal bone with Sevriton. One needle was connected to a constant flow perfusion pump through a polyethylene tubing, and through this end Ringer was pumped into the dialysis tube. From the other needle the Ringer was collected into a mini-tube fitted on the Eppendorf tip. Although the Ringer, because of its superficial tension, tends to remain at the tip of the mini-tube, a plastic diaphragm was inserted at the base of the needle to prevent dropping of the sample. A schematic diagram of the whole system is shown in Figure 1. After the end of the implantation the rat's skin was sutured, the area was infiltrated with Novocaine, the anesthesia was discontinued, and the rat was allowed to recover and to have free access to water and food. Normally, we allowed a 24 hr recovery before any experiment was performed. Thus, the day after the implantation the rat was transferred into hemispheric bowl of 50 cm diameter and the inlet to the dialysis tube was connected to the pump by polyethylene tubing with the interposition of a liquid swivel; this swivel has the function of avoiding the twisting of the polyethylene tube following the animal's movement in the bowl. Although we built the liquid swivel ourselves, a fully adequate one can be purchased from Harvard Instrument Co. The swinging part of the liquid swivel is connected through rigid aluminum wires to a blanket tailored around the animal's body so that turning of the rat will twist the aluminum wire and let turn the mobile part of the swivel.

Analytical procedure. As in the previous study (Imperato and Di Chiara, 1984), the dialysis tube was perfused with Ringer at a speed of 2 μ l/min, and samples were collected every 10 or 20 min after placing 5 or 10 μ l of 0.1 N perchloric acid at the tip of the mini-tube. The samples were then directly injected into an HPLC equipped with reverse-phase columns and with an electrochemical detector in order to separate and quantitate in a single run DA, DOPAC, and HVA. The apparatus, the operating conditions, and the mobile phase used are exactly the same as those already described for anesthetized rats (Imperato and Di Chiara, 1984).

Estimation of catalepsy. Catalepsy was estimated by placing the rats on a vertical grid and recording the time during which the rats remained immobile with the four limbs extended on the grid. A score was assigned as follows: score 1 (15 sec), score 2 (30 sec), score 3 (60 sec).

Results

Basal output of DA and metabolites. Figure 2 shows the time course of the output of DA, DOPAC, and HVA starting 1 hr after the implantation of the dialysis tube up to 24 hr after. Figure 3 shows the time course during the subsequent 4 days. This time course was characterized by an initial phase, during the first 6 hr, when DA output decreased while that of DOPAC

Figure 1. Schematic diagram of a trans-striatal dialysis preparation in an awake rat.

Figure 2. Time course of DA, DOPAC, and HVA output from 1 to 24 hr after the implantation of the dialysis tube. Results are expressed as picomoles in 20-min samples (40 μ l of dialysate). Results are means $\pm$ SEM of five rats.

Figure 3. Time course of DA, DOPAC, and HVA output from 24 hr to 4 days after the implantation. Results are expressed as picomoles in 20-min samples (40 μ l). Results are means $\pm$ SEM of five rats.

and HVA increased. The second phase was characterized by a very slow decrease of DA, DOPAC, and HVA during the following 4 days. During this period DA, DOPAC, and HVA output decreased by about 35%.

Histology. Figure 4 shows the histological appearance of the caudate nucleus of a rat implanted 4 days earlier with the dialysis tube and fixed by intracardiac formalin during the dialysis procedure. The hole left by the dialysis tube (about 0.2 mm diameter) and the normal architecture of the surrounding tissue can be seen. No signs of demyelination, inflammation, or thrombosis are observed. The track left by the dialysis tube is bordered by a palisade of radially ordered ependyma-like glia surrounded by a thin, loose wall of fibrous glia.

Adjacent to this wall, scattered pyknotic neurons can be seen

among normally appearing perikarya. Beyond this point, the histological and cytological characteristics of the tissue are normal.

Systemic haloperidol, flupentixol, and sulpiride. Subcutaneous administration of haloperidol (0.012 to 0.5 mg/kg) produced a dose-related catalepsy and an increase of DA release and of DOPAC and HVA output. Figures 5 to 8 show the time course of the effects of doses of 0.012, 0.025, 0.1, and 0.5 mg/kg, s.c., of haloperidol. Threshold doses for these effects were around 0.012 mg/kg, s.c. (Fig. 5), which increased by a maximum of 40% the release of DA. Maximal increase of DA release was obtained after doses of 0.25 mg/kg of haloperidol, but the increase was not higher than a doubling of basal values. Increasing the dose of haloperidol prolonged the duration of the

Figure 4. Histology of the caudate nucleus of a rat implanted with the dialysis tube 4 days earlier. Luxol-fast hematoxylin stain. A, Sagittal section through the caudate (magnification $\times 25$) showing the location of the track left by the dialysis tube cut transversally. B, The track left by the tube showing a palisade of endepidyma-like glia surrounded by a loose wall of fibrous glia (magnification $\times 100$).

increase of DA release. Also, the peak increase of DOPAC and HVA was related to the dose of haloperidol. After 0.5 mg/kg of haloperidol (Fig. 8), a 3- to 3.5-fold increase of DOPAC and HVA output was obtained. The increase of DOPAC output was faster than that of HVA, but the magnitude of the increase at the plateau was similar for the two metabolites. The time course of the stimulatory effect of haloperidol on DA release did not

coincide with that on DOPAC and HVA output. DA release always returned to basal values earlier than DOPAC and HVA output. For example, after doses of 0.1 mg/kg (Fig. 7) of haloperidol, DA release was back to basal values at a time (3 hr) when DOPAC and HVA were in plateau and when catalepsy was maximal.

Subcutaneous administration of *cis*-flupentixol stimulated

Figure 5. Time course of DA, DOPAC, and HVA output after 0.012 mg/kg, s.c., of haloperidol. Results are expressed as percentage of basal values and are means $\pm$ SEM of four rats. Basal values (picomoles $\pm$ SEM) were: DA, 0.29 ± 0.03 ; DOPAC, 38 ± 3.7 ; HVA, 30 ± 3.8 . *, $p < 0.05$ in respect to basal values.

Figure 6. Time course of DA, DOPAC, and HVA output after 0.025 mg/kg, s.c., of haloperidol. Results are expressed as percentage of basal values and are means $\pm$ SEM of four rats. Basal values (picomoles $\pm$ SEM) were: DA, 0.32 ± 0.03 ; DOPAC, 44 ± 4.8 ; HVA, 35 ± 4.2 . *, $p < 0.05$ in respect to basal values. Catalepsy is expressed as mean score $\pm$ SEM.

DA release and DOPAC and HVA output in a manner very similar to that of haloperidol. Threshold doses for stimulation of DA release and DOPAC and HVA output were around 0.025 mg/kg, s.c. After the highest doses tested (0.25 and 1.0 mg/kg, s.c.), DA release was not stimulated by more than 100% whereas DOPAC and HVA increased maximally by about 300% (data not shown). As in the case of haloperidol, DA release returned to basal values 2 or 3 hr before the start of recovery of DOPAC and HVA toward basal values. After 0.05 or 0.25 mg/kg, s.c., of *cis*-flupentixol, the intensity of catalepsy was still maximal at a time when DA release had returned to basal values. *trans*-Flupentixol at a dose of 5 mg/kg, s.c., had an effect similar to 0.1 mg/kg of the *cis*-form (data not shown).

(-)-Sulpiride, the most active enantiomer of sulpiride (Lafayette and Margarit, 1968; Tagliamonte et al., 1975; Mielke et al., 1977) as a DA receptor antagonist (Spano et al., 1979), stimulated DA release and metabolism at doses of 2.5 to 100 mg/kg, s.c.

The intensity and the duration of the stimulation of DA release were dependent upon the dose administered but did not exceed, as after haloperidol, a doubling of the basal values. DOPAC and HVA output also increased in a dose-related manner, the increase of DOPAC being faster than that of HVA. As shown in Figure 9, after doses of 10 mg/kg, s.c., of (-)-sulpiride, the maximal increase of DA release was by about 50% over basal values at 1 hr and lasted about 3 hr, whereas that of DOPAC and HVA lasted for about 6 hr. As shown in Figure 10, after 100 mg/kg, s.c., of (-)-sulpiride, while DA release was stimulated by 100%, the maximal output of DOPAC and HVA increased by more than 200% in respect to basal values. When the time course of DA release is compared with that of DOPAC and HVA output, one can still note that DA release tends to come back to basal values earlier than DOPAC and even more so when compared to HVA output.

(-)-Sulpiride at all the doses tested failed to produce catalepsy. Doses of 10 to 50 mg/kg, s.c., resulted in an initial period of hyperactivity, followed by sedation, whereas doses of 100 mg/kg, s.c., produced only sedation. (+)-Sulpiride produced a significant increase of DA release and DOPAC and HVA output only at doses of 100 mg/kg, s.c.

Interaction of γ -butyrolactone with neuroleptics. In order to evaluate the role of dopaminergic firing activity for the effect of neuroleptics on DA release and metabolism, the interaction of haloperidol (0.1 mg/kg, s.c.) and sulpiride (20 mg/kg, s.c.) with γ -butyrolactone was investigated. Figure 11 shows the time course of the effect of 20 mg/kg of sulpiride on DA release and DOPAC and HVA output. After this dose of sulpiride, DA release was maximally stimulated by about 80% 40 min after administration and remained at these high levels for about 2 hr and then slowly declined toward basal values. DOPAC and HVA output increased maximally by about 250% 60 min and 100 min after sulpiride and remained in plateau up to 5 hr after administration. As shown in Figure 12, administration of γ -butyrolactone (200 mg/kg, i.p.) 1 hr after sulpiride rapidly reversed the stimulatory effect of sulpiride on DA release. DA release was already back to basal values 20 min after γ -butyrolactone and after 1 hr was about 40% lower than basal values; about 2 hr after γ -butyrolactone DA release rapidly recovered to the values observed with sulpiride alone.

In contrast with its effects on DA release, γ -butyrolactone potentiated the stimulation of DOPAC and HVA output produced by sulpiride; thus, after γ -butyrolactone, DOPAC and HVA further increased, reaching a new level of about 300 to 350% of the basal one. As shown in Figure 13, γ -butyrolactone (200 mg/kg, s.c.) given alone reduced within 40 min basal DA release by about 50% with a recovery within 3 hr. DOPAC and HVA output remained unchanged. The effect of γ -butyrolactone (200 mg/kg, i.p.), given 1 hr after 0.1 mg/kg, s.c., of

Figure 7. Time course of DA, DOPAC, and HVA output after 0.1 mg/kg, s.c., of haloperidol. Results, expressed as percentage of basal values, are means $\pm$ SEM of five rats. Basal values (picomoles $\pm$ SEM) were: DA, 0.33 ± 0.04 ; DOPAC, 35 ± 4.2 ; HVA, 28 ± 3.2 . *, $p < 0.05$ in respect to basal values. Catalepsy is expressed as mean score $\pm$ SEM.

Figure 8. Time course of DA, DOPAC, and HVA output after 0.5 mg/kg, s.c., of haloperidol. Results, expressed as percentage of basal values, are means $\pm$ SEM of four rats. Basal values (picomoles $\pm$ SEM) were: DA, 0.35 ± 0.04 ; DOPAC, 46 ± 5.2 ; HVA, 37 ± 4.3 . *, $p < 0.05$ in respect to basal values. Catalepsy is expressed as mean score $\pm$ SEM.

Figure 9. Time course of DA, DOPAC, and HVA output after 10 mg/kg, s.c., of (-)-sulpiride. Results, expressed as percentage of basal values, are means $\pm$ SEM of four rats. Basal values were: DA, 0.26 ± 0.03 ; DOPAC, 35 ± 4.0 ; HVA, 30 ± 3.0 . *, $p < 0.05$ in respect to basal values.

haloperidol was almost superimposable to that just described for sulpiride and is not shown.

Discussion

The present study provides a method to study DA release in the awake rat adapted from the previous one applied by us to halothane-anesthetized rats (Imperato and Di Chiara, 1984). Such a method is based on the principle of dialysis. By this principle, low molecular weight substances present outside of 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 flowing inside it. A major feature of this method is its relatively minor ability to produce local trauma, mainly due to the lack of direct physical contact of the perfusion fluid with the nervous tissue, its small diameter (0.2 mm outer diameter), and its softness (acrylic copolymer). This characteristic is confirmed by the present study, showing that 4 days after the implantation of the dialysis tube into the brain, no sign of inflammation, infarction, or distortion of the surrounding tissue are present. The only reaction to the dialysis tube is a layer of ependyma-like glia around the tube surrounded by a thin, loose wall of fibrous glia. The almost geometric order of the first wall of glia and the loose appearance of the second one testifies to the minimal amount of mechanical trauma exerted by the dialysis tube on the surrounding tissue.

The second characteristic of the present method which should be underlined is the fact that DA release and DOPAC and HVA output can be estimated for at least 4 days from the implantation of the dialysis tube. After the first 6 hr, during which relatively rapid changes take place, the DA release and metabolite output are so stable within a 24-hr period that even long-lasting experiments can be performed with accuracy and reliability. With our method, drugs like haloperidol, sulpiride, and flupentixol, which are chemically different and have a widely different spectrum of receptor activities but have in common the ability to block dopaminergic D-2 receptors, also share the ability to stimulate the release of DA. This action is stereospecific as the (-)-sulpiride and *cis*-flupentixol, which

Figure 10. Time course of DA, DOPAC, and HVA output after 100 mg/kg, s.c., of (-)-sulpiride. Results, expressed as percentage of basal values, are means $\pm$ SEM of five rats. Basal values were: DA, 0.26 ± 0.03 ; DOPAC, 39 ± 4.5 ; HVA, 33 ± 3.8 . *, $p < 0.05$ in respect to basal values.

are the most active isomers as neuroleptics, are at least 10 times more active in stimulating DA release *in vivo* than the corresponding (+)- or *trans*- forms (Miller et al., 1974; Enna et al., 1976; Spano et al., 1979); moreover, this effect is produced by doses of the effective neuroleptics which are in the same range as those of the most sensitive tests of neuroleptic activity *in vivo*. This effect is apparently associated with the classically known property of neuroleptics to stimulate DA metabolism, a property which is expressed in our system by an increased output of DOPAC and HVA. However, some important differences between the effect of neuroleptics on DA release and that on DA metabolism are observed. First, there is a discrepancy in the time course of DA release and that of metabolite output. In fact, DA release returns to basal values 2 to 3 hr before DOPAC and HVA output starts to decrease toward basal values. It is unlikely that this difference is due to a change in the relative permeability of the dialysis tube to DA, DOPAC and HVA since no change was found when the permeability was tested *in vitro* 24 hr after the *in vivo* implantation of the tube (A. Imperato and G. Di Chiara, manuscript in preparation).

The discrepancy in the time course of DA release and metabolite output is not due to a slower kinetics of the recovery of DOPAC and HVA from the tissue by the dialysis tube since the duration of the plateau phase of the stimulatory effect on metabolite output is also time related to the behavioral effects of neuroleptics (sedation and catalepsy). Thus, after high doses of neuroleptics, DA release is back to basal values at a time when the behavioral effects of neuroleptics (sedation and catalepsy) are still maximal. This observation indicates that,

Figure 11. Effect of 20 mg/kg of (-)-sulpiride s.c. on DA, DOPAC, and HVA output. Results, expressed as percentage of basal values, are means $\pm$ SEM of four rats. Basal values were: DA, 0.31 ± 0.03 ; DOPAC, 40 ± 4.3 ; HVA, 36 ± 3.2 . *, $p < 0.05$ in respect to basal values.

although the stimulation of DA release by neuroleptics is triggered by the blockade of DA receptors, this change recovers in spite of continuous blockade of DA receptors. On this basis we suggest that the mechanism which, triggered by a blockade of DA receptors, leads to stimulation of DA release, undergoes some kind of rapid tolerance. In agreement with this suggestion, we have observed (A. Imperato and G. Di Chiara, manuscript in preparation) that administration of a second 0.1 mg/kg (s.c.) dose of haloperidol immediately after the return of DA release to basal values fails to stimulate DA release while it prolongs the plateau phase of DOPAC and HVA output. In contrast, the stimulatory effect of neuroleptics on DA metabolism does not appear to be affected by the rapid tolerance which affects the stimulation of DA release. Such discrepancy in the time course of DA release and metabolism calls for a different mechanism of the two effects. A working hypothesis which explains the present data postulates that the neuroleptic-induced stimulation of DOPAC and HVA output, which represents their stimulatory action on DA synthesis, is mediated by blockade of terminal DA autoreceptors inhibitory on DA synthesis (Kehr et al., 1972; Carlsson, 1975; Di Chiara et al., 1977). Such an effect is a local phenomenon, unrelated to the firing activity of DA neurons. 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 located in the striatum (Carlsson and Lindqvist, 1963; Bunney and Aghajanian, 1976) or on nigral DA receptors postsynaptic to DA

Figure 12. Time course of DA, DOPAC, and HVA output after administration of (-)-sulpiride (20 mg/kg, s.c.) followed by γ -butyrolactone (200 mg/kg, i.p.) 1 hr later. Results, expressed as percentage of basal values, are means $\pm$ SEM of four rats. Basal values were: DA, 0.28 ± 0.02 ; DOPAC, 42 ± 4.0 ; HVA, 27 ± 3.0 . *, $p < 0.05$ in respect to basal values.

dendrites and located on striatonigral terminals (Phillipson and Horn, 1976; Spano et al., 1976, 1977; Gale et al., 1977). In the light of this hypothesis, the rapid recovery of neuroleptic-induced stimulation of DA release might simply reflect a rapid recovery of the stimulation of DA firing. Unfortunately, however, there is no published complete time course of the effect of neuroleptics on DA firing, and, therefore, our hypothesis will have to wait for such information in order to be tested. The dose response curve for the stimulation of release appears strikingly similar to that for firing and, like DA release, also, DA firing is doubled after maximally effective doses of neuroleptics. Again, these observations are in agreement with the possibility that stimulation of DA release after neuroleptics results from stimulation of DA firing.

The discrepancy between DA release and DA metabolism is in line with previously expressed views on the regulatory mechanisms of DA neurons. Many instances are known in which certain indices of dopaminergic activity are differentially affected; for example, although stimulation of DA synthesis *in vivo* and activation of tyrosine hydroxylase (TH) are often associated, γ -butyrolactone provides an example of dissociation between the two, since it markedly stimulates DA synthesis *in*

Figure 13. Effect of γ -butyrolactone (GBL) (200 mg/kg, i.p.) on DA, DOPAC, and HVA output. Results, expressed as percentage of basal values, are means $\pm$ SEM of four rats. Basal values were: DA, 0.33 ± 0.03 ; DOPAC, 37 ± 3.8 ; HVA, 33 ± 3.5 . *, $p < 0.05$ in respect to basal values.

in vivo (Walters et al., 1973) but fails to activate TH and actually prevents the activation of TH produced by neuroleptics (Zivkovic et al., 1975). γ -Butyrolactone, on the other hand, is known to turn off the firing activity of DA neurons (Walters et al., 1973) and, as we have recently demonstrated, drastically reduces DA release *in vivo* (Imperato and Di Chiara, 1984). Another example of this kind is provided by kainate lesions of the striatum, which prevent the ability of neuroleptics to activate TH activity (Di Chiara et al., 1978) and to stimulate DA firing (Kondo and Iwatsubo, 1980) but not the ability to stimulate DA synthesis and metabolism *in vivo* (Di Chiara et al., 1978).

Compelling evidence for the concept that, after neuroleptics, DA release and firing activity are related but are distinct from DA synthesis and metabolism is provided by the interaction between γ -butyrolactone and neuroleptics like sulpiride and haloperidol. In this case impairment of dopaminergic firing by γ -butyrolactone reverses the haloperidol and sulpiride-induced stimulation of DA release while it potentiates the stimulation of DOPAC and HVA output. It is unlikely that the effect of γ -butyrolactone on the neuroleptic-induced stimulation of DOPAC and HVA output is due to impairment of the efflux of the acids from the brain, since the dose of γ -butyrolactone is too low to produce this effect; on the other hand, this dose of γ -butyrolactone fails to modify, when given alone, DOPAC and HVA output. The potentiation of DOPAC and HVA output could be due to the fact that the combined action of blockade of DA autoreceptors by the neuroleptic and reduction of DA release by γ -butyrolactone results in complete deactivation of DA autoreceptors. We interpret these results to signify that stimulation of dopaminergic firing activity is essential for the neuroleptic-induced stimulation of DA release, and that removal of the DA input on terminal DA autoreceptors is insufficient for stimulating DA release but efficiently and powerfully stimulates DA metabolism and synthesis. This does not mean that terminal DA autoreceptors do not control DA release; in fact, there is still the possibility that their blockade by neuro-

leptics plays a permissive role for the stimulation of DA release by dopaminergic firing activity. In other words, we cannot exclude the possibility that the neuroleptic-induced stimulation of DA release is related to the combination of stimulation of DA firing and blockade of terminal DA autoreceptors. Indeed, recent findings with the novel neuroleptic Sch 23390, which is a poor antagonist of DA autoreceptors, is in line with this possibility (G. Di Chiara and A. Imperato, manuscript in preparation).

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