Dopamine in substantia nigra and cortex after γ-butyrolactone treatment

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Until recently it was assumed that dopamine terminals were present only in the basal ganglia, subcortical limbic system and hypothalamus. The cerebral cortex appeared to possess exclusively noradrenergic nerve terminals originating in the locus coeruleus (Fuxe, Hamberger & Hökfelt, 1968; Ungerstedt, 1971). However, Thierry and coworkers (Thierry, Stinus & others, 1973a; Thierry, Blanc & others, 1973b) have provided substantial evidence for the existence of cortical dopamine nerve terminals. Such terminals are found in the frontal cortex, anterior cingulate cortex, the ventral part of the entorhinal cortex and in pyriform cortex (Lindvall, Björklund & others, 1974; Hökfelt, Fuxe & others, 1974a; Hökfelt, Ljungdahl & others, 1974b; Berger, Tassin & others, 1974). The results of lesion experiments suggest that the projection to the frontal cortex originates in the A10 cell area of the ventral tegmentum whereas the projection to the anterior cingulate cortex arises from dopamine cell bodies situated in the lateral substantia nigra (Lindvall & others, 1974).

It also has been suggested, on the basis of histochemical studies, that the fluorescent varicosities observed on dopaminergic processes extending into the pars reticulata of the substantia nigra indicate the presence of dopaminergic dendrodendritic synaptic endings in this part of the rat brain as well (Björklund & Lindvall, 1975). Similar fluorescent processes have been seen within zona compacta and pars lateralis of the substantia nigra in the rhesus monkey (Sladek & Parnavelas, 1975).

Among the drugs which can be used to manipulate dopaminergic function is γ-hydroxybutyric acid (GHB) and also its precursor γ-butyrolactone (GBL). GHB has been shown to inhibit the firing of the dopamine neurons in the pars compacta of the substantia nigra (Walters, Aghajanian & Roth, 1972; Roth, Walters & Aghajanian, 1973) and the ventral tegmental area (Walters, Aghajanian & Roth, unpublished observations). A decrease in dopaminergic impulse flow caused either by administration of GHB, GBL, or by lesion of the nigro-neostriatal pathway causes an acute and substantial increase in neostriatal dopamine concentration (Gessa, Vargiu & others, 1966; Aghajanian & Roth, 1970; Walters & Roth, 1972; Andén, Magnusson & Stock, 1973; Stock, Magnusson & Andén, 1973). A number of techniques have shown that this change in dopamine concentration is associated with an increase in dopamine synthesis (Spano, Tagliamonte & others, 1971; Carlsson, Kehr & others, 1972; Roth & others, 1973; Agid, Javoy & Glowinski, 1974; Walters & Roth, 1974; Kehr, 1974). Dopamine agonists are able to block or reverse the increase in dopamine synthesis associated with blockade of impulse flow in the nigro-neostriatal pathway (Kehr, Carlsson & others, 1972; Roth & others, 1973; Walters & Roth, 1974, 1976). It has been suggested that dopamine receptors located on the presynaptic side of the dopamine terminal may be involved with the modulation of dopamine synthesis and release and that the effects of the agonists on dopamine synthesis after inhibition of impulse flow are due to an interaction with these presynaptic receptors (Kehr & others, 1972; Roth & others, 1973; Walters & Roth, 1974, 1976).

In view of the foregoing observations it was of interest to determine whether GBL and presynaptic receptor stimulation affects dopamine concentrations in the regions containing dopamine cell bodies and newly described terminals.

Male, Sprague-Dawley rats (Zivic-Miller Labs), 170–230 g were used. GBL (Cole-Matheson & Bell) was administered instead of GHB, because it is more lipid soluble and more easily absorbed. In the blood, GBL is rapidly metabolized to GHB which is the active form of the drug. GBL, apomorphine HCl (Merck) and 1-(2-pyrimidyl)-piperonyl-piperazine (ET-495, Servier Laboratories) were administered intraperitoneally. The animals were decapitated, and each brain with its ventral surface up, placed immediately in a brain slicer over an ice-cooled plate. Beginning from the anterior part of frontal lobe, transversal slices (1 mm thick) were made with razor blades, and cortical pellets were punched out of the appropriate slice with a metal tube, internal diameter 1·5 mm. The frontal cortex punch was obtained from the ventral part of the second slice, about 1·5 mm from the medial line, just above the genu of the corpus callosum, and tissue pellets of the cingulate cortex were punched out from the ventral medial cortex of the fourth slice at the level of the anterior commissure.

The anterior border of the pons was used as a landmark for dissection of substantia nigra. The first blade was put at that level, while the second one was placed 2 mm in front of the first and a metal tube, 0·5 mm internal diameter, was used to obtain a punch from the tissue ventral to the medial lemniscus. Ventral tegmental area containing A10 cells was punched from the triangle between the interpenduncular nucleus, medial lemniscus and substantia nigra with a metal tube, 1·5 mm internal diameter. The tissue was homogenized immediately in 400 μl 0·1 N $HClO_4$. Protein concentration was determined in 10 μl of homogenate according to Lowry, Rosebrough & others (1951), and dopamine and adrenaline were assayed by micro-

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Table 1. *Regional effects of γ-butyrolactone* on dopamine concentrations (ng mg^{-1} protein).*

	Nucleus Caudate	Ventral Tegmental area	Substantia nigra
Control	95 ± 7	11·4 ± 0·9	7·34 ± 0·97
GBL	190 ± 8**	11·3 ± 0·8	7·29 ± 0·26

* γ-Butyrolactone (750 mg kg^{-1}, i.p.) was administered to rats 60 min before death. The results are mean ±s.e.m. of 6 experiments.
** $P<0·001$ *vs* control.

modification of a sensitive enzymatic isotopic method (Coyle & Henry, 1973; Palkovits, Brownstein & others, 1974). Dopamine in the samples was measured by correlation with corresponding tissue standards which were run in each assay.

Dopamine concentrations in the ventral tegmental area and substantia nigra were not changed after GBL treatment, although there were marked elevations in the caudate nucleus (Table 1). Quite different results were obtained in the cerebral cortex. Frontal and anterior cingulate tissue pellets had significantly increased concentrations of dopamine after GBL treatment (Fig. 1). However, the cortical increase of dopamine induced by GBL was not so pronounced as in neostriatum (179% of control in frontal cortex and 157% in cingulate cortex compared to 251% in the neostriatum). While noradrenaline concentrations after GBL treatment were not changed in the cingulate cortex, they decreased significantly in frontal cortex (Table 2). The administration of the dopamine receptor agonists, apomorphine or ET-495, before GBL treatment, prevented the effect of GBL on dopamine concentrations in both parts of the cortex (Fig. 1), but did not change the concentration of noradrenaline in these regions (Table 2).

Our data show that inhibition of impulse flow in dopamine neurons has two different effects on dopamine terminals in cerebral cortex and on dopamine cell bodies and newly hypothesized dopamine dendritic terminals in the substantia nigra. These results suggest that the increase in dopamine synthesis following inhibition of impulse flow occurs only in axon terminals, although the possibility that increased synthesis of dopamine in nigral and ventral tegmental areas is accompanied by increased degradation cannot be excluded. The fact that dopamine concentrations did not increase in the ventral tegmental area and substantia nigra after GBL treatment is in good agreement with fluorescent studies of Aghajanian & Roth (1970). These authors have described increased fluorescence of neostriatal dopamine nerve terminals in GBL-treated rats, but they did not observe any change in the nerve cell bodies.

The recent hypothesis that dopamine neurons might make synapses in the substantia nigra (Björklund & Lindvall, 1975) has many interesting implications for the site of action of drugs influencing dopaminergic transmission. This idea has obtained additional support with the demonstration of dopamine sensitive adenylate cyclase in this part of the brain (Kebabian & Saavedra, personal communication). However, our data do not provide evidence for existence of such synapses in substantia nigra, but they do suggest that if dopaminergic dendritic terminals are there, they function differently from neostriatal dopamine axon terminals.

On the other hand, our attention has been directed to the cortex. Of the regions receiving dopaminergic innervation, the cortical and mesolimbic areas are thought the most likely sites of therapeutic drug action in schizophrenia (Hökfelt & others, 1974b). Many studies have shown that neuroleptic drugs block dopamine receptors in the neostriatum and mesolimbic areas and cause a compensatory increase in synthesis and release of dopamine in these regions (Carlsson & Lindqvist, 1963; Wilk, Watson & Glick, 1975; Creese, Burt & Snyder, 1975). Fluorescent techniques have provided evidence that pimozide increases turnover of cortical dopamine (Fuxe, Hökfelt & others, 1975a). Scatton, Thierry & others (1975) have also shown that treatment with phenothiazines results in an increase in dopamine synthesis in the cerebral cortex, although, interestingly, these workers found that higher concentrations of neuroleptics were required to produce these effects in the cortex than in the neostriatum. Since GBL decreases the release of dopamine and the activity of dopamine neurons and consequently leads to decreased concentrations of dopamine competing for the postsynaptic receptor, it has been suggested that this drug could be used as an adjuvant, combined with neuroleptics, in the treatment of schizophrenia (Fuxe, Agnati & others, 1975b). On the other hand, it should be pointed out that GBL itself increases dopamine synthesis which might limit its usefulness in this regard. As the present study shows, GBL administration can increase dopamine concentrations in the frontal and anterior cingulate cortex, although not to the same extent as in the neostriatum. However, it is

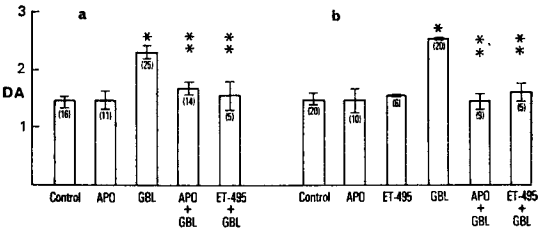

FIG. 1. The effect of γ-butyrolactone, apomorphine and ET-495 on dopamine (DA) concentration (ng mg^{-1} protein) in **a**-cingulate and **b**-frontal cortex. The results are mean value ± s.e.m. Numbers in parentheses represent number of experiments. Apomorphine (2 mg kg^{-1}) and ET-495 (10 mg kg^{-1}) were injected intraperitoneally 70 min before death. GBL (750 mg kg^{-1}) was administered intraperitoneally 60 min before death. * Significantly different from control $P<0·001$. ** Significantly different from GBL treated group $P<0·001$.

Table 2. *The effect of apomorphine, ET-495 and γ-butyrolactone on noradrenaline concentrations in cingulate and frontal cortex.*

Treatment	Noradrenaline (ng mg^{-1} protein) Cingulate cortex	n	Frontal cortex	n
Control	2·64 ± 0·20	16	2·48 ± 0·19	10
Apo	2·53 ± 0·26	9	2·33 ± 0·08	5
ET-495			2·05 ± 0·22	4
GBL	2·56 ± 0·10	25	1·72 ± 0·15*	10
Apo + GBL	2·27 ± 0·17	14	2·34 ± 0·21	4
ET-495 + GBL			2·14 ± 0·23	5

Apomorphine (2 mg kg^{-1}) and ET-495 (10 mg kg^{-1}) were injected intraperitoneally 70 min before death. γ-Butyrolactone (750 mg kg^{-1}) was administered intraperitoneally 60 min before death. The results are the mean ±s.e.m. of n experiments.
* $P<0.01$ *vs* control.

difficult to predict from these acute studies how combined chronic treatment with low doses of GBL and a neuroleptic would affect dopamine synthesis and release in the various brain regions receiving dopamine input.

The GBL-induced increase in dopamine concentrations was blocked in both cortical areas with dopamine receptor agonists. This finding is similar to results reported for the neostriatum (Walters & Roth, 1974). When impulse flow is prevented, apomorphine appears to inhibit dopamine synthesis and elicit changes in dopamine turnover by stimulation of presynaptic dopaminergic receptors with consequent inhibition of the tyrosine hydroxylase activity (Kehr & others, 1972; Roth & others, 1973; Ebstein, Roberge & others, 1974; Westfall, Besson & others, 1976). Such an inhibition of tyrosine hydroxylase may occur also in the cortical areas, where the activation of presynaptic dopamine receptors presumably takes place after the impulse flow in the dopamine neurons projecting to the cortex has been inhibited by GBL administration. This drug has also caused a significant drop in noradrenaline concentrations in frontal cortex but not in the cingulate cortex. The functional importance of this change is not known at present.

In conclusion, changes in dopamine concentrations similar to those observed in the neostriatum occur in the frontal and cingulate cortex after blockade of dopaminergic impulse flow and treatment with dopamine agonists. However, regulation of dopamine synthesis in the dopamine cell bodies of the substantia nigra and ventral tegmental area and in the region of the recently hypothesized nigra dendritic terminals differs from that described in the neostriatum.

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The effects of blocking catecholamine uptake on amphetamine-induced circling behaviour in mice with unilateral destruction of striatal dopaminergic nerve terminals

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A number of tricyclic compounds used clinically as antidepressant agents are believed to function by blocking the uptake of released central neurotransmitters into their respective presynaptic nerve terminals. Thus, since re-uptake is an important mechanism for the inactivation of released substance, more transmitter will be available in the synaptic cleft for receptor interaction. Tricyclic antidepressants are used commonly in patients with Parkinson's disease, and have been shown to produce additional modest improvement in motor function (Strang, 1965). This may be due to their inherent antiacetylcholine properties, or, alternatively, to their capacity to block re-uptake of released dopamine in surviving nigrostriatal terminals. To investigate the latter possibility we have studied the effect of blocking the uptake of the catecholamines noradrenaline and dopamine on the dopamine-dependent circling behaviour in mice with unilateral destruction of one nigrostriatal dopamine pathway. In such animals the directly acting dopamine agonist, apomorphine, causes turning towards the intact side, due, it is suggested, to the preferential stimulation of supersensitive denervated striatal dopamine receptors, while indirectly acting dopamine agonists, such as amphetamine, cause circling towards the lesioned side, due to release of endogenous dopamine from the intact nigrostriatal terminals (Ungerstedt, 1971).

Unilateral destruction of nigrostriatal dopamine nerve terminals in mice was achieved by the free-hand injection of 16 μg 6-hydroxydopamine in 4 μl chilled saline containing 0·2 mg ml^{-1} ascorbic acid into the right striatum as previously described (von Voigtlander & Moore, 1973; Pycock, Tarsy & Marsden, 1975). Ten days later those animals showing tight ipsiversive circling to amphetamine (5 mg kg^{-1}, i.p.) and strong contraversive circling to apomorphine (2 mg kg^{-1}, i.p.) were selected for the following series of experiments to test the effect of pretreatment of catecholamine uptake blocking agents on amphetamine-induced circling behaviour. A complete Latin square design was used to randomize the distribution of nomifensine, amantadine, desipramine, amitriptyline and benztropine to groups of 10 mice, including a control series of saline-injected animals. At the end of 30 min, when the effects were maximal, the mice were observed for any circling behaviour or postural asymmetries produced by the blocking drugs. After such assessments the mice were injected with amphetamine (1·5 mg kg^{-1}, i.p.), a dose that causes submaximal rates of turning (Pycock & others, 1975). Thirty min after amphetamine administration, the number of full circles completed by each animal was counted and compared with the rate of circling in the control saline-treated groups. Mice were tested in this way to various doses of blocking agent on alternate days so that at least 10 observations for each dose of drug was obtained.

Of the five uptake inhibitors tested in this mouse model only nomifensine and benztropine produced circling behaviour. Nomifensine, in the dose range 5–40 mg kg^{-1}, caused a mild ipsilateral postural asymmetry and ipsiversive rotational behaviour in a dose graded response (Fig. 1A) confirming the observation of Costall, Kelly & Naylor (1975). Benztropine in the dose range 1·5–50 mg kg^{-1}, similarly caused some ipsilateral body posturing together with circling towards

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