CHOLINERGIC–DOPAMINERGIC RELATION IN DIFFERENT BRAIN STRUCTURES

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NEOSTRIATAL neurons respond in an opposite manner to the iontophoretic application of dopaminergic and cholinergic compounds and to their respective antagonists. Thus, dopamine (DA) depresses, whereas acetylcholine (ACh) or physostigmine enhance the firing rate of caudate cells (BLOOM *et al.*, 1965). Facilitation by physostigmine is not only inhibited by atropine but also by L-DOPA (STEG, 1969). Depletion of striatal DA (e.g. by reserpine) also increases the discharge frequency of striatal units (STEG, 1969). It seems, therefore, that at least some striatal cells receive antagonistic cholinergic (activating) and dopaminergic (inhibitory) inputs.

This antagonism probably results in a functional balance determining the efferent motor control of the striatum (BARBEAU, 1962). Unbalance towards a cholinergic preponderance—as for example due to impaired dopaminergic transmission—leads to parkinsonian symptoms. Several observations support this concept of a functional balance between antagonistic dopaminergic and cholinergic activities. Thus, cholinergic drugs (e.g. physostigmine) exacerbate (DUVOISIN, 1967), anticholinergic compounds, however, ameliorate parkinsonism (SIGWALD, 1971). In addition, anticholinergic drugs counteract the parkinsonism induced by neuroleptic blockade of dopaminergic transmission in both animals (ZETLER *et al.*, 1960) and man (KLAWANS, 1968). Finally, L-DOPA, which restores the dopaminergic activity, has an outstanding therapeutic effect in parkinsonian patients whose striatal DA is greatly reduced (Lit. see HORNYKIEWICZ, 1972). The question which arises is whether or not such a functional balance implies an interconnection between striatal dopaminergic and cholinergic neurons resulting in a mutual regulation.

It is likely that the striatal dopaminergic neurons are regulated by a cholinergic system. Thus, physostigmine enhances (PEREZ-CROUET *et al.*, 1971), whereas anticholinergic compounds diminish the turnover of cerebral DA (BARTHOLINI and PLETSCHER, 1971). A possible interpretation of these findings is that an increase or decrease in cholinergic activity leads to a compensatory activation or inhibition of DA neurons respectively, suggesting a link between the two systems. However, no direct evidence exists for this view; there is even less indication for the inverse regulation of cholinergic neurons by the DA system.

A direct approach to the problem of the interconnection and the mutual regulation of striatal DA and ACh systems is provided by the measurement of drug-induced changes in the amounts of transmitters continuously released within the striatum. This has been made possible by means of a push–pull cannula implanted into the head of the caudate nucleus of the gallamine immobilized cat. ACh released into the perfusate was measured radioenzymatically in 10 min samples collected throughout several hours (STADLER *et al.*, 1973). The output of ACh was markedly increased by neuroleptic drugs such as chlorpromazine (CPZ) (Fig. 1, Table 1) or haloperidol

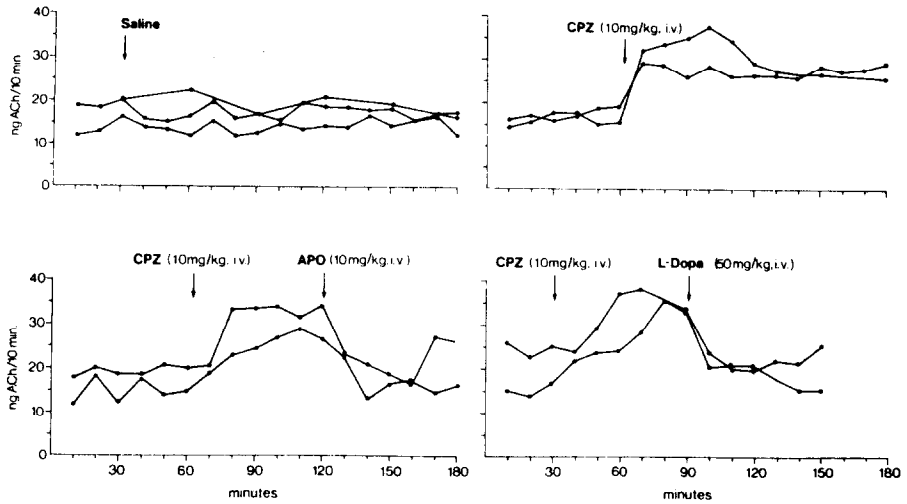

FIG. 1.—Output of acetylcholine (ACh) into 10 min samples of the perfusate of cat caudate nucleus during the control (preinjection) period and after various treatments. Each curve indicates one experiment. The points represent averages of duplicate determinations. Time 0 indicates the beginning of collection.
CPZ = chlorpromazine; APO = apomorphine.

(Table 2). This effect of CPZ is not due to a non specific interaction with gallamine since similar data were obtained in chronically implanted, unrestrained cats (STADLER *et al.*, 1973). The increase in ACh output seems to result from enhanced turnover since in preliminary experiments the concentration of ACh was not changed by CPZ in the perfused caudate nucleus of two cats (controls: 2·46; CPZ: 2·63 ng/mg tissue). Inhibition of ACh-esterase (acetylcholine acetyl-hydrolase, EC3.1.1.7) by CPZ can be excluded as the enzyme activity was unaffected in striatal tissue (controls: 40·5; CPZ: 41·9 mU/mg tissue). Neither is the enhanced output of ACh by CPZ specific for the phenothiazine configuration since promethazine, a non-neuroleptic phenothiazine with antihistaminic properties, did not modify the ACh release during 2 hr perfusions (Table 1) (STADLER *et al.*, 1973). In preliminary experiments atropine (25 mg/kg i.v.) increased the striatal ACh output in agreement with similar findings in the cortex (MITCHELL, 1963; PEPEU, 1971). However, for CPZ its anticholinergic properties can be at most partially responsible for the increased ACh release since also haloperidol, a neuroleptic butyrophenone devoid of both anticholinergic and antihistaminic properties, markedly enhanced the output of the transmitter.

It is therefore likely that the enhancement of striatal ACh release by neuroleptics is connected with the blockade of DA receptors by these drugs. This hypothesis is supported by the fact that apomorphine—a DA receptor stimulating compound (ANDÉN *et al.*, 1966)—or L-DOPA injected 1 hr after CPZ reversed the neuroleptic-induced increase in ACh output (Fig. 1). In addition, apomorphine alone diminished the release of ACh and, injected before CPZ, prevented the enhancement of the transmitter due to the latter drug (Table 1). Finally, the enhancement of ACh release by CPZ or haloperidol in the perfused cat striatum occurred concomitantly to the acceleration of DA turnover as evidenced by the rise of homovanillic acid (Table 2). This effect of neuroleptics on DA turnover (ANDÉN *et al.*, 1964), which

TABLE 1. RELEASE OF ACETYLCHOLINE (ACh) INTO THE PERFUSATE OF THE CAT CAUDATE NUCLEUS. The ACh contents of the 10-min samples (ng/10 min) collected throughout the time periods indicated were averaged and statistically evaluated by the Student's t test. Saline, chlorpromazine and promethazine when given alone, were injected at 60 min. In the combined treatment apomorphine was administered at 60 min followed by chlorpromazine at 120 min. The samples collected from 60 to 70 and 120 to 130 min were omitted. In parentheses: at left number of determinations, at right number of cats. *$P < 0.001$ vs control period.

Treatment (mg/kg i.v.)	Time after beginning of collection (min)		
	control	post injection	
	0–60	70–120	130–180
Saline	14·5 ± 0·6 (12) (2)	15·2 ± 0·7 (10) (2)	15·8 ± 0·6 (10) (2)
Chlorpromazine (10)	16·2 ± 0·9 (30) (5)	25·5 ± 0·8* (25) (5)	26·3 ± 1·2* (20) (4)
Promethazine (10)	15·1 ± 1·1 (12) (2)	14·6 ± 0·6 (10) (2)	16·3 ± 1·1 (10) (2)
Apomorphine (10) followed by chlorpromazine (10)	14·1 ± 0·42 (12) (2)	10·6 ± 0·74* (12) (2)	13·72 ± 1·4 (12) (2)

probably results from the blockade of DA receptors, did not occur after promethazine (unpublished results) which also failed to enhance ACh output (see above).

All of the above-mentioned results indicate that a striatal cholinergic system may be under a regulatory dopaminergic influence (BARTHOLINI *et al.*, 1973). It is possible that the nigro-striatal DA pathway modulates the activity and function of some cholinergic neurons by a tonic inhibitory input. Thus, impairment of dopaminergic transmission would result in an increased activity of cholinergic neurons, ACh release and in the

TABLE 2. CONCENTRATIONS OF DOPAMINE (DA), HOMOVANILLIC ACID (HVA) IN THE CAT CAUDATE NUCLEUS AND OF ACETYLCHOLINE (ACh) IN THE PERFUSATE. DA and HVA have been measured in the perfused caudate nucleus of the cat 2 hr after injection of saline or of the drugs. The values for ACh in perfusate represent the averages with SEM of the concentrations in 10 min samples collected during the second hour after injection. In parentheses at left number of determinations, at right number of cats. *$P < 0.01$ vs saline treated animals.

Treatment (mg/kg i.v.)	Caudate nucleus		Perfusate
	DA (μg/g)	HVA (μg/g)	ACh (ng/10 min)
Saline	11·97 ± 0·51 (15) (15)	3·94 ± 20 (15) (15)	15·8 ± 0·6 (12) (2)
Chlorpromazine (10)	12·03 ± 1·11 (6) (6)	5·87 ± 0·62* (7) (7)	26·3 ± 1·2* (24) (4)
Haloperidol (2)	9·04 ± 0·30 (12) (12)	6·13 ± 0·51* (12) (12)	35·2 ± 4·7* (12) (2)

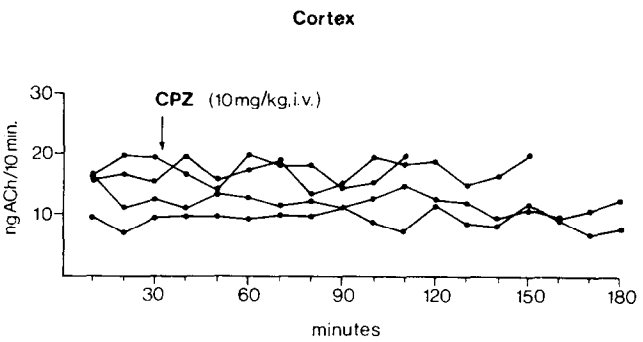

FIG. 2.—Output of acetylcholine (ACh) into 10 min samples of the perfusate of cat pre-motor cortex during the control period (0–30) and after chlorpromazine (CPZ). For details see Fig. 1.

appearance of parkinsonian symptoms. This is supported by the observations that anticholinergic drugs ameliorate rigidity and tremor in Parkinson's disease and extrapyramidal dysfunction caused by neuroleptic compounds (see above).

Based on these findings it must be assumed that the regulation of a striatal cholinergic activity by dopaminergic neurons occurs via by a link between DA and ACh systems. The possibility that dopaminergic neurons act on the cholinergic system by a presynaptic input should be considered.

In conclusion, it is likely that in the neostriatum dopaminergic and cholinergic neurons are interconnected and mutually regulating in such a way that cholinergic activity is under an inhibitory dopaminergic influence whereas the DA system is activated by a cholinergic input:

$$DA \underset{+}{\overset{-}{\rightleftarrows}} ACh$$

This interregulation might explain the mechanism by which, in the striatum, DA turnover is enhanced as a consequence of neuroleptic blockade of DA receptors. Thus, impairment of dopaminergic transmission activates a cholinergic system which in turn would cause a compensatory increase in the activity of DA neurons.

The question arises whether or not such a link between DA and ACh neurons exists in areas of the brain other than the striatum. In our cat preparation neuroleptics did not change the ACh release from the perfused pre-motor cortex (Fig. 2). Other areas were also investigated, for instance limbic structures, which may be of particular interest for the therapeutic effect of neuroleptic drugs. In fact, it has been hypothesized that blockade of DA receptors in the limbic system could be involved in the antipsychotic action of neuroleptics. In our experiments, haloperidol—although it markedly enhanced the DA turnover—did not modify the release of ACh from the nucleus accumbens septi—is the limbic region with the most dense DA network. This suggests that, as for cortical areas, a cholinergic-dopaminergic connection similar to that in the striatum does not exist in the nucleus accumbens septi of the cat and that the antipsychotic action of neuroleptic drugs may not be mediated by cholinergic neurons in this structure. For details on the effect of neuroleptic drugs on limbic DA and ACh neurons see: LLOYD *et al.* (this book).

SUMMARY

The influence of the dopamine (DA) neurons on a cholinergic system in the neostriatum has been investigated in experiments in which acetylcholine (ACh) liberated from the cat caudate nucleus was collected by means of a push-pull cannula and measured radioenzymatically. Neuroleptic drugs (e.g. chlorpromazine or haloperidol, i.v.) enhanced the output of striatal ACh and this effect was prevented or reversed by dopaminergic agents such as apomorphine or L-DOPA. Neuroleptics also increased DA turnover. Promethazine, a non-neuroleptic phenothiazine, did not affect either ACh or DA. From these and other findings it is concluded that in the neostriatum a cholinergic mechanism, which might be involved in parkinsonism, is under the influence of a tonic dopaminergic inhibitory input whereas the DA system is activated by cholinergic neurons. This mechanism is possibly involved in the feed-back regulation of DA turnover. As neuroleptics failed to affect ACh output in cortex and limbic structures—although in the latter these drugs markedly increased DA turnover—such a link and mutual regulation between DA and ACh systems seems to be specific for the neostriatum.

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