γ-Aminobutyric acid: the essential mediator of behaviour triggered by neostriatally applied apomorphine and haloperidol

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There is now good evidence that γ-aminobutyric acid (GABA) functions as a neurotransmitter in the central nervous system (Roberts, 1974). Based on the facts that (a) GABA occurs within both the neostriatum and the substantia nigra (Robinson & Wells, 1973), (b) the GABA-content of the nigra decreases following axotomy of striato-nigral fibres (Kim, Bak & others, 1971), and (c) electrical stimulation of the neostriatum produces an inhibitory effect on nigral cells (Yoshida & Precht, 1971), it is generally accepted that there exists a gabaminergic, striato-nigral fibre system, which is inhibitory in nature. Studies analysing the effect of aminooxyacetic acid, a GABA-transaminase inhibitor, on the striatal dopamine content have, in addition, indicated that these fibres may function as a part of a mechanism controlling the nerve impulse flow in certain dopaminergic, nigro-neostriatal fibres (Stock, Magnusson & Andén, 1973).

The fact that haloperidol, a potent dopamine-receptor antagonist, inhibits the GABA-uptake (Iversen & Johnston, 1971) and increases the GABA-turnover (Collins, 1973) cannot be explained on the basis of this type of interaction between the neuronal systems. Recent electrophysiological studies, however, have suggested that there are possibly also gabaminergic fibres, which arise from the substantia nigra and terminate within the neostriatum (Feltz, 1974); indeed, GABA is not only present within intracaudate terminal structures, but it is also able to produce postsynaptic inhibitions in caudate units, which are selectively counteracted by GABA-antagonists such as picrotoxin and bicuculline (Feltz, 1974; Robinson & Wells, 1973).

In the present work the mutual dependency between intracaudate gabaminergic and dopaminergic mechanisms has been studied at the functional level by determining behavioural changes brought about by single or combined injections of dopamine, apomorphine, haloperidol, GABA and picrotoxin into the caudate nucleus of cats. Since dopamine produces the so-called contralateral syndrome when administered into the caput nuclei caudati rostromedialis (CRM) and the so-called ipsilateral syndrome when administered into intracaudate structures surrounding this area (r-CRM) (Cools, Janssen & others, 1975) these two regions have been analysed separately.

Adult, male cats, 2·5–3·5 kg, were stereotaxically equipped with cannulae implanted into the CRM and r-CRM according to previously described methods (Cools & van Rossum, 1970). Experiments were initiated one week after surgery by which time the cats showed normal placing responses, righting and pupillary reflexes, food-intake and reactivity to visual and acoustic stimuli. Drug-solutions (5 μl) were injected through injection-needles which extended into the brain tissue 2 mm below the tip of the cannula. Dopamine HCl, apomorphine HCl, picrotoxin, GABA—dissolved in distilled water and adjusted to pH 4·5—and haloperidol (Serenase, Janssen Pharmaceutica, Belgium) were freshly prepared before each experiment. The behaviour recorded on videotape by means of a closed circuit TV was analysed according to Cools & Janssen (1974). Thus, the number of *forced* head movements directed towards each side of the body were counted during 15 min immediately following the injection. Scores indicating the preferential side were given as follows: NT = absence of any turning; BT = absence of any turning preference; IT = ipsilateral head movements, i.e. movements directed towards the injected side exceeding 75%

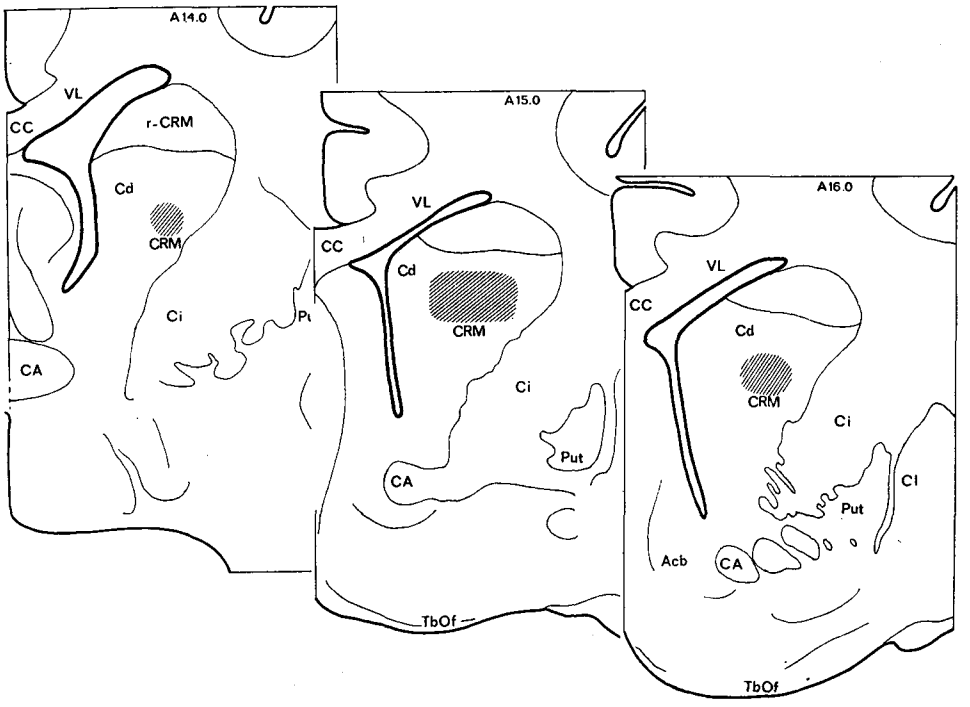

FIG. 1. Semi-diagrammatic outline of three anterior frontal planes in the basal ganglia of cats, showing the CT-inducing dopamine-sensitive area (CRM) and the IT-inducing dopamine-sensitive area (r-CRM). Acb = nucleus accumbens; CA = commisura anterior; CC = corpus callosum; Cd = nucleus caudatus; Ci = capsula interna; Cl = nucleus centrolateralis thalami; Put = putamen; TbOf = tuberculum olfactorium; Vl = ventriculus lateralis.

of the total frequency of these movements; and CT = contralateral head movements, i.e. movements directed towards the non-injected side exceeding 75% of the total frequency of the induced head movements. Since injections of distilled water or the vehicle for haloperidol remained ineffective, the behaviour displayed during a period of 15 min immediately preceding the injection was used as control: animals showing forced head-movements during the blank test were discarded. Each drug or drug-combination was tested in at least seven injection-loci; the number of injections per cannula was restricted to eight. After completion of the experiments the identification of the injection-sites was determined by reference to the atlas of Snider & Niemer (1964) as described by Cools & van Rossum (1970). The data presented below are derived from experiments in which the injection-sites were restricted to the areas in Fig. 1.

The main results are summarized in Tables 1 and 2. The data concerning dopamine, apomorphine and haloperidol have confirmed the outcome of previously reported experiments (Cools & others, 1975): apomorphine locally applied into the CRM-area mainly produced CT, whereas when it was applied into the r-CM-area produced either CT, NT or BT; haloperidol locally applied into the CRM-area produced only IT, whereas when applied into the r-CRM-area produced either IT, NT or BT. GABA (10 μg) locally applied either into the CRM- or the r-CRM-area produced IT. Since the effect was an "all or none" response at an individual-specific concentration, it was not possible to study the dose-effect relationship in a normal way. Therefore, several doses in different animals were tested to determine a dose which

produced the GABA-response in more than 75% of the sites tested: a dose of 10 μg fulfilled this requirement (Table 1). Doses of 7 and 5 μg only resulted in IT in about 30% of the tested sites (n = 17), whereas a dose of 2 μg GABA remained ineffective in about 77% of the sites tested (n = 17). As the cats were not derived from a standard stock, it remains to be elucidated whether or not genetic characteristics have contributed to this steep dose-response curve. Picrotoxin (5 μg) locally applied either into the CRM- or the r-CRM-area produced CT in all sites tested (n = 31); doses of 2 μg produced CT in about 39% of the tested sites (n = 31). Lower doses of picrotoxin were not given (Table 1). In general, all compounds produced their effects after a latency of about 2–5 min, whereas the response itself lasted about 7–12 min; the strongest effect was always observed after a latency of about 8 min.

When dopamine (10 μg) was administered into the CRM-area of animals locally pretreated 5 min earlier with haloperidol (12 μg), the dopamine-induced IT was completely suppressed in 12 cases (n = 13); previously, it has been shown that higher doses of dopamine can reverse this effect (Cools, 1971). Haloperidol did not affect the IT elicited by dopamine in 10 cases (n = 12). When haloperidol (12 μg) was given 5 min before apomorphine (0·6 μg) into the CRM-area, the apomorphine-induced CT was suppressed in 10 cases (n = 13). Haloperidol affected the NT or BT elicited by apomorphine in a complex way: animals showing BT after a single injection of apomorphine displayed CT following the haloperidol-apomorphine treatment, whereas those animals, which showed NT after a single injection of apomorphine displayed BT following the haloperidol-apomorphine treatment. Apart from the data shown in Table 2, it has been found that the CT elicited by apomorphine administration into this area was completely suppressed by the haloperidol pretreatment. When GABA (10 μg) was administered into the CRM- or r-CRM-area of animals locally pretreated

Table 1. *Turning preference of head movements produced by unilateral injections of apomorphine, haloperidol, γ-aminobutyric acid (GABA) and picrotoxin into distinct, dopamine-sensitive sites related to the effectiveness of dopamine administered at identical sites into the caput nuclei caudati rostromedialis (CRM-area) and intracaudate structures surrounding the CRM-area (r-CRM-area) of cats.* CT = contralateral turning; IT = ipsilateral turning; NT = no turning; BT = bilateral turning; x = not tested.

	Dopamine-induced turning preference[c]							
	CRM-area[a]				r-CRM-area[b]			
Turning compounds (μg)	CT n = 17				IT n = 14			
	CT	IT	NT	BT	CT	IT	NT	BT
Apomorphine (0·6)	14	—	—	3	5	—	4	5
Haloperidol (12)	—	17	—	—	—	2	3	9
GABA (2)	—	4	5	8	—	2	5	7
(5)	—	4	1	12	x	x	x	x
(7)	—	5	—	12	x	x	x	x
(10)	—	17	—	—	—	14	—	—
Picrotoxin (2)	6	—	—	11	6	—	—	8
(5)	17	—	—	—	14	—	—	—

[a] Chemical stimulation of 4 sites did not result in CT after dopamine injections into the CRM-area (n = 21).

[b] Chemical stimulation of 8 sites did not result in IT after dopamine injections into the r-CRM-area (n = 22).

[c] Animals with dopamine-sensitive sites either ineffective in producing CT within the CRM-area or ineffective in producing IT within the r-CRM-area were discarded.

Table 2. *Turning preference of head movements produced by unilateral injections of dopamine, apomorphine, GABA and picrotoxin into distinct, dopamine-sensitive sites within the caput nuclei caudati of cats with and without a local pretreatment with various agents; these effects are related to the effectiveness of dopamine (DA) administered at identical sites.* CT = contralateral turning; IT = ipsilateral turning; NT = no turning; BT = bilateral turning.

Turning compounds (μg)	Contralateral syndrome inducing DA-sites n = 13				Ipsilateral syndrome inducing DA-sites n = 12			
	CT	IT	NT	BT	CT	IT	NT	BT
Haloperidol[a] (12) Dopamine (10)	—	12	—	1	—	10	—	2
Haloperidol[a] (12) Apomorphine (0·6)	—	10	—	3	5[b]	—[b]	—[b]	4[b]
Picrotoxin[a] (5) GABA (10)	13	—	—	—	12	—	—	—
		n = 9				n = 11		
Dopamine[a] (10) GABA (10)	—	8	—	1	—	11	—	—
Apomorphine[a] (0·6) GABA (10)	—	9	—	—	—	10	—	1
Haloperidol[a] (12) Picrotoxin (5)	9	—	—	—	8	2	—	1

[a] This compound was given 5 min before the second injection.
[b] Only animals showing NT (n = 4) or BT (n = 5) after a single injection of either haloperidol or apomorphine were tested.

5 min earlier with picrotoxin (5 μg), the GABA-induced IT was completely suppressed.

As shown in Table 2 GABA (10 μg) locally applied into the CRM-area 5 min after the administration of dopamine (10 μg) or apomorphine (0·6 μg) into the same site completely suppressed the CT induced in 8 and 9 cases respectively (n = 9); picrotoxin (5 μg) locally administered into the CRM-area surmounted the IT-induced by haloperidol (12 μg), although the combination of these substances resulted in the additional appearance of convulsions (n = 9). Neither dopamine (10 μg) nor apomorphine (0·6 μg) affected the IT elicited by the administration of GABA (10 μg) into the r-CRM-area. Furthermore, pretreatment with haloperidol (12 μg) did not affect the CT produced by local administration of picrotoxin (5 μg) into the r-CRM-area.

The present findings clearly show that neostriatal GABA-sensitive sites are indispensable links in the process triggered by the dopamine-agonist apomorphine and antagonist haloperidol at those sites of which dopamine-activation results in the elicitation of CT: in fact, effects either produced by apomorphine and dopamine or by haloperidol are completely suppressed by GABA and the GABA-antagonist picrotoxin respectively. A different relation appears to exist between GABA and those sites at which dopamine-activation results in the elicitation of IT: then, both dopamine and GABA produced identical effects.

In view of the facts that (a) haloperidol does increase the GABA turnover (Collins, 1973), (b) haloperidol locally applied into the CRM-area produces effects identical to those elicited by GABA, and (c) the GABA-antagonist picrotoxin completely inhibits the effects induced by haloperidol, it can be postulated that blockade of the dopamine-sensitive sites within the CRM-area disinhibits the GABA-mechanism. The observa-

tions that (a) apomorphine or dopamine locally applied into the CRM-area produced effects which are diametrically opposite to those elicited by GABA, but identical to those induced by picrotoxin, and (b) GABA completely suppressed the effects induced by dopamine or apomorphine are in agreement with this hypothesis.

Recent studies have shown that the dopamine-antagonists haloperidol and apomorphine also interfere with dopamine-sensitive mechanisms presynaptically localized (Christiansen & Squires, 1974; Kehr, Carlsson & others, 1972; Seemann, 1974). From this point of view, it is attractive to postulate that the release of GABA is, in fact, modulated by the degree of stimulation of dopamine-sensitive sites presynaptically localized on gabaminergic neurons (see also: Cools & van Rossum, 1975); this speculation, however, awaits further experimentation. The present data show that the intracaudate dopaminergic system is able to modulate a gabaminergic system within this nucleus; in other words, the activity within certain nigro-neostriatal, dopaminergic fibres is under control of certain striato-nigral, gabaminergic fibres (Stock & others, 1973), whereas the activity in gabaminergic mechanisms within the caudate nucleus is, in turn, under control of dopaminergic systems.

As a final remark, it is important to stress that the caudate nucleus contains two distinct types of dopamine-sensitive sites as discussed by Cools & van Rossum (1975): the so-called excitation-mediating types, which are stimulated by dopamine and apomorphine, inhibited by haloperidol, and unaffected by ergometrine, noradrenaline and 1-(2-pyrimidyl)-piperonyl-piperazine (ET-495) (DA_e-types), and the so-called inhibition-mediating types, which are stimulated by dopamine and (3,4-dihydroxyphenylamino)-2-imidazoline (St-1943), inhibited by ergometrine, noradrenaline and ET-495, but not affected by low doses of apomorphine or haloperidol (DA_i-types) (Cools & others, 1975; Cools, 1975). Since the DA_e types are mainly concentrated within the CRM-area in contrast to the DA_i-types, which are diffusely distributed throughout the r-CRM-area, the present findings indicate that especially the DA_e types are involved in the modulation of the neostriatal GABA-mechanisms.

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