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Muscimol and related GABA receptor agonists: the potency of GABAergic drugs in vivo determined after intranigral injection

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Contralateral turning behaviour following unilateral intranigral injection of a large series of GABA analogues was investigated. The results indicated that the turning behaviour was induced stereospecifically and was selectively antagonized by the GABA antagonist bicuculline methochloride. The comparative potencies of a series of GABA agonists related to muscimol in general corresponded well to the affinity for ^{3}H-GABA receptor sites and to the depressant action on single neurons using microelectrophoretic administration. However, the GABA agonists *trans*-aminocrotonic acid and 3-aminopropanesulphonic acid were much weaker than expected from in vitro studies. The GABA-uptake inhibitors nipecotic acid and guvacine showed only weak and short-lasting effects. The GABA-transaminase inhibitor γ-acetylenic GABA showed delayed effects compared with the agonists which acted immediately. It is proposed that this behavioural effect may be a sensitive and quantitative method for evaluation of GABA agonists in vivo.

Unilateral injection of γ-aminobutyric acid (GABA) into the caudal part of substantia nigra, pars reticulata (SNR), induces a strong contralateral turning in rats (Scheel-Krüger et al 1977). This effect was mimicked by three GABA-ergic drugs: muscimol, baclofen and imidazole acetic acid. Conversely the GABA antagonists picrotoxin and bicuculline methiodide induced ipsilateral turning. Similar results have been found by Olpe et al (1977), Oberlander et al (1977), Waddington (1977) and Olianas et al (1978).

The contralateral turning behaviour, which was dose-dependent and induced by a wide range of doses of the different drugs, seems to represent a useful in vivo model for determinations of relative potencies of putative GABA agonists. The only direct in vivo model available is based on micro-electrophoretic application of compounds on single neurons (Krogsgaard-Larsen et al 1975). However, this electrophysiological technique provides only semi-quantitative information of the potency of the compounds and provides limited information on the time-course of action in vivo.

The results obtained after unilateral injection of a large series of GABA agonists and related compounds into the substantia nigra was compared with published results obtained from microelectrophoretic experiments and data from GABA receptor binding studies in vitro (Enna & Snyder 1977; Krogsgaard-Larsen 1978; Krogsgaard-Larsen & Johnston 1978; Olsen et al 1978).

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MATERIALS AND METHODS

Surgery and experimental schedule. Male Wistar SPF rats (180–200 g) were anaesthetized with pentobarbitone (50–55 mg kg^{-1}, i.p.) and implanted with stainless steel guide cannulae vertically above substantia nigra (SN), aimed at the coordinates A 1·6 L 2·1 (König & Klippel 1963). The guide cannulae terminated 2 mm dorsal to SN. The rats were allowed at least one week for recovery and had free access to food pellets and water until the experiments began. Unilateral intranigral injections of drugs in 1 μl were made into hand-restrained conscious rats over 30–45 s using a 31 G Hamilton microsyringe (o.d. 0·25 mm). The syringe was held in position for further 30 s. In the experiments using combined muscimol and bicuculline methochloride (BMC) treatment, muscimol was injected 25 min before BMC, both drugs being in a volume of 0·5 μl. Immediately after the injection, the rats were placed individually in large open areas (50 × 75 cm) for continuous behavioural observation. After the experiments the rats were perfused through the heart with 4% formaldehyde (50 ml) under deep pentobarbitone anaesthesia. The brains were removed, fixed for at least one week, sectioned in

40 μm slices, stained with cresylviolet and examined for the exact injection site. Only injections within the area determined by the coordinates A 1·27–1·95 L 1·7–2·4 and V ÷ 2·6–3·0 (the ventral part of pars reticulata) were included in the results.

Evaluation of the results. The contralateral turning was expressed as the maximum frequency during 1 min (rev min^{-1}) and was found within 1–20 min after injection, dependent on the drug. Since the maximum frequency of rotation for most of the drugs was approximately 25 rev min^{-1}, an ED 50 value (expressed as nanomoles injected) was defined as the dose corresponding to 12·5 rev min^{-1}. For each drug, 2–5 doses were used to calculate ED 50 values by means of log-probit analysis (Finney 1952). As the highest acceptable dose we used 50 μg. At least 4 experiments were made at each dose level. The antagonism of muscimol by BMC was expressed as the % reduction in turning frequency for each rat. Statistical significances were calculated by Student's *t*-test.

Drugs. The following drugs were all synthesized at the laboratory of Dr P. Krogsgaard-Larsen: muscimol, THIP, HBr (4,5,6,7-tetrahydroisoxazolo [5,4-c] pyridin-3-ol), (*RS*)-5′-methylmuscimol, (*R*)-(+)-5′-methylmuscimol,(*S*)-(−)-5′-methylmuscimol, homomuscimol, HBr, isoguvacine, HBr, azamuscimol, 2HCl, thiomuscimol, 4-methylmuscimol, HBr, isomuscimol, 5′-ethylmuscimol, (*S*)-(−)-*trans*-4-aminopent-2-enecarboxylic acid (methyl-t-ACA), (4*S*, 3 *RS*)-(−)-4-amino-3-hydroxy-pentanecarboxylic acid (methyl-GABOB), *R*-(−)-nipecotic acid and guvacine. We acknowledge the gifts of 3-aminopropanesulphonic acid (3-APS), *trans*-4-aminocrotonic acid (t-ACA) and bicuculline methochloride (BMC) from Dr G. A. R. Johnston, Canberra, Australia and the gift of γ-acetylenic GABA (GAG) from Centre de Recherce Merrell International, France. Sodium 4-hydroxybutyrate, piperidine-4-carboxylic acid (isonipecotic acid) and ethanolamine-*O*-sulphate (EOS) were obtained from commercial suppliers. All optically active compounds were tested as racemates unless configurations are stated. Formulae are given in the Tables. Solutions with drug concentrations below 5 μg μl^{-1} were made in saline, whereas more concentrated solutions were made in bidistilled water, in order to reduce very high osmotic pressure. Control injections revealed no differences between saline or water as solvent. All solutions were, if necessary, adjusted to neutral pH. Doses of salts expressed in μg were corrected to the amount of active substance.

RESULTS

Unilateral intranigral injection of saline or water did not induce significant turning on any occasion ($n > 10$). The contralateral turning response after GABA agonists was critically dependent on the localization of the cannula tip. Injections of GABA agonists just caudal or dorsal to SNR were ineffective, whereas injections in the rostral part of SNR resulted in lower activity often with a time latency of 10–15 min.

Potency of GABA agonists and related compounds. The dose response curves for contralateral turning induced by the series of GABA agonists, muscimol, THIP, isoguvacine, methyl-t-ACA and t-ACA are shown in Fig. 1. Following doses near the threshold (i.e. muscimol 5 ng; THIP, 160 ng) turning behaviour

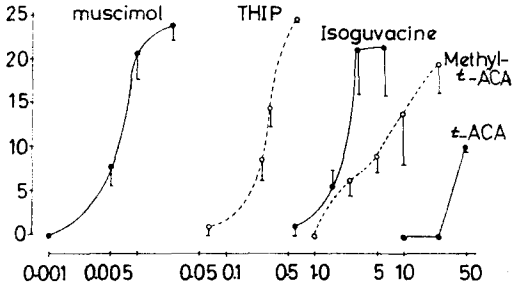


FIG. 1. Contralateral turning behaviour after unilateral injection of GABA agonists into the caudal substantia nigra, zona reticulata, in rats. Abscissa: μg injected in 1 μl. Ordinate: maximum turning frequency (rev min^{-1}). Each value represents the mean ± s.e.m. of at least 4 experiments.

was often accompanied by increased motility and rearing all around the field but turning was always contralateral. Rats injected with higher doses rotated in a tight head-to-tail manner with only episodical locomotor activity. These rats showed intense stereotyped head movements and sniffing. Supramaximal doses (not shown) often reduced turning frequency probably caused by the profound stereotypy which dominated the behaviour of the rats. The slopes of the dose response curves depicted in Fig. 1 were typical for most of the compounds. Only homomuscimol and thiomuscimol had flat curves similar to that of methyl-t-ACA.

The contralateral turning was stereospecifically induced as demonstrated by injection of (*R*)- and (*S*)-5′-methylmuscimol (Fig. 2). The more active (*S*)-enantiomer was not significantly different from the racemate but the (*R*)-enantiomer was about 20 times less active in agreement with the findings in receptor affinity binding studies (Table 1).

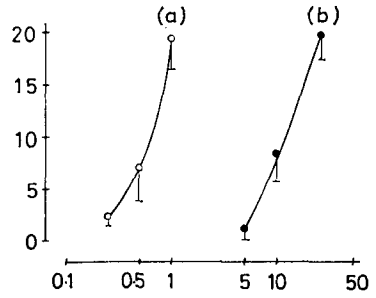


FIG. 2. Stereospecific induction of contralateral turning behaviour after unilateral injection of (*S*)-(−)-5′-methylmuscimol (a) (*R*)-(+)-5′-methylmuscimol (b) into the substantia nigra. Abscissa: μg injected in 1μl. Ordinate: maximum turning frequency (rev min^{-1}). Each value represents the mean ± s.e.m. of 5–7 experiments.

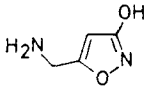
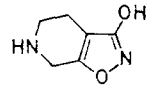
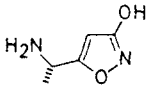
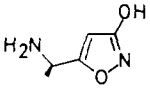
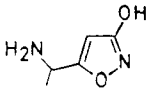
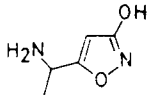
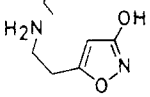
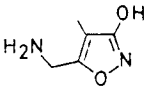
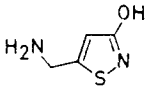
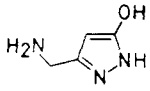
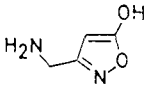
The comparative potencies of a series of muscimol analogues are presented in Table 1. ED 50 values for contralateral turning are compared with published data on ^{3}H-GABA receptor affinity binding experiments (IC 50 values). Minor modifications in molecular structure resulted in dramatic decreases in potency or eventually loss of activity of the compounds. This was found after substitution in or elongation of the aliphatic side chain in muscimol as illustrated with the series: muscimol, 5′-methylmuscimol and 5′-ethylmuscimol (ED 50 values: 0·06, 5·6 n mol and inactive, respectively), and muscimol and homomuscimol (ED 50 values: 0·06 and 6·7 n mol). Similarly, substitution in the heterocyclic nucleus (4-methylmuscimol) and alterations of the structure of the heterocyclic ring of muscimol (thiomuscimol, azamuscimol and isomuscimol) reduced the potency.

The comparative potencies of a series of GABA agonists structurally different from muscimol are presented in Table 2 together with the potency of some GABA-uptake and GABA-transaminase (GABA-T) inhibitors. These GABA agonists were much weaker compounds, in particular the non-cyclic amino acids t-ACA (ED 50: 560 n mol) and 3-APS (ED 50: 284 nmol) compared with receptor affinity binding (Table 2; see also Fig. 1).

The GABA uptake inhibitors *R*-(−)-nipecotic acid and guvacine both induced a shortlasting turning response following extremely high intranigral doses (nipecotic acid, 50 μg: 22 ± 10 turns min^{-1}, duration 13 min; guvacine, 50 μg: 12 ± 6 turns min^{-1}, duration 6 min).

The GABA-T inhibitors EOS and GAG were also able to induce contralateral turning, EOS being comparable with the weakest GABA agonists with respect to potency and duration (50 μg: 6 ± 1·5 turns min^{-1}, duration 15 min). In contrast, GAG was completely different in the time course of action (Fig. 3): immediately after the injection of 40 μg into SNR, contralateral turning with low frequency was

Table 1. Comparative potency of muscimol analogues obtained from intranigral injection and ^{3}H-GABA receptor binding studies. The potency after intranigral injection is given as the amount (in nmol) inducing half-maximal turning response.

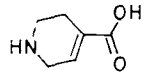
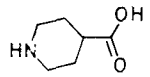
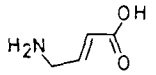
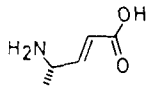
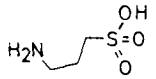
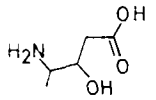
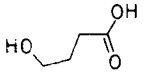
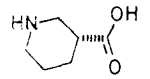
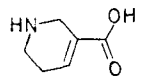
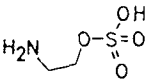
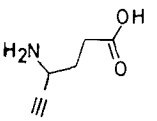
Compound	Structure	Turning response ED 50 (nmol)*		^{3}H-GABA binding‡ IC 50 (μmol)
Muscimol		0·06	(0·04–0·10)	0·024 ± 0·003
THIP		1·7	(1·0–2·8)	2·6 ± 0·5
S-(−)-5′-methyl-muscimol		5·1	(3·2–7·3)	0·64 ± 0·07†
R-(+)-5′-methyl-muscimol		113	(68–186)	19 ± 1†
RS-5′-methyl-muscimol		5·6	(3·5–9·0)	2·2 ± 0·1†
RS-5′-ethyl-muscimol		0		0
Homo-muscimol		6·7	(0·8–57)	10 ± 2
4-methyl-muscimol		64	(39–105)	>100
Thio-muscimol		28	(13–60)	0·12 ± 0·01
Azamusci-mol		36	(23–57)	>100
Isomusci-mol		276	(175–436)	377 ± 55

* 95% confidence limits in brackets.
† Krogsgaard-Larsen et al (1978).
‡ Krogsgaard-Larsen (1978).

observed for a few min followed by 10–15 min without effects. After 15–20 min turning behaviour started again and gradually increased until the maximal effect was observed 60–80 min after injection. The turning response never reached the high frequency typical for the agonists. Spontaneous turning was present for at least 3 h and some rats showed contralateral asymmetry after 24 h.

The turning response and central stimulation was similar for all active compounds with one exception, methyl-t-ACA. Relatively much more pronounced stereotyped behaviour was observed after injection of this compound with subsequently slower rotation frequency.

Table 2. Comparative potency of GABA analogues obtained from intranigral injection and ^{3}H-GABA receptor binding studies. The potency after intranigral injection is given as the amount (in nmol) inducing half-maximal turning response.

Compound	Structure	Turning response ED 50 (nmol)[1]		^{3}H-GABA binding IC 50 (μmol)
Isoguvacine		17	(10–29)	1·4 ± 0·1*
Isonipecotic acid		471	(61–3647)	15 ± 2*
t-ACA		560	(263–1191)	0·2†
S-(−)-methyl-t-ACA		66	(27–163)	4·1 ± 0·4‡
3-APS		284	(200–405)	0·3†
Methyl-GABOB		210	(99–447)	200 ± 15‡
4-Hydroxybutyrate		>397		n.t.
R-(−)-Nipecotic acid		276	(194–393)	0*
Guvacine		403	(235–691)	0*
EOS		>357		n.t.
RS-GAG		292	(158–544)	n.t.

[1] 95% confidence limits in brackets.
* Krogsgaard-Larsen & Johnston (1978).
† Enna & Snyder (1977).
‡ Honoré et al (1978)..
n.t. = not tested.

The time course of action. The time course of action for muscimol and thiomuscimol is presented in Fig. 3. Muscimol and thiomuscimol showed clear dose dependency also with regard to duration of action. Doubling of duration required approximately a 5 times higher dose (muscimol 5 and 25 ng; thiomuscimol 5 and 25 μg). An important difference between muscimol and thiomuscimol appeared from the duration of action: although both compounds were immediately acting, muscimol induced the maximum turning frequency after 10–20 min. This level was maintained for the next 20–40 min after a dose of 25 ng, followed by a slow decline in activity. In contrast, thiomuscimol after both low or high doses induced the maximum effect immediately after injection and the effect faded quickly off. This type of time course of action was found for few of the drugs: t-ACA (6 min), isonipecotic acid (10 min), methyl-GABOB (6 min), nipecotic acid (13 min) and guvacine (6 min) (Table 3). In Table 3 comparative data on the duration of all the compounds studied are summarized.

Isoguvacine showed an extremely long-lasting effect, more than 3–6 h following injection of 3–6 μg, whereas 0·6 μg was inactive. Homomuscimol showed an anomalous dose-duration profile; from very short-lasting effects after 0·6 μg (20 min), the duration time was increased 5-fold by injection of 3 μg.

Time course aspects for GABA uptake and GABA-T inhibitors has been mentioned in the potency section above.

Inhibition by selective GABA-antagonists of muscimol induced turning. The muscimol induced turning was significantly reduced by the GABA antagonist BMC 25 and 100 ng, intranigrally (Fig. 4, $P < 0·05$). BMC was injected 25 min after muscimol when a stable turning frequency had been established (Fig. 3). Decrease in turning frequency was observed 2–3 min after BMC injection and the antagonism lasted 15–30 min, dependent on the dose of BMC. Later the turning again increased indicating the

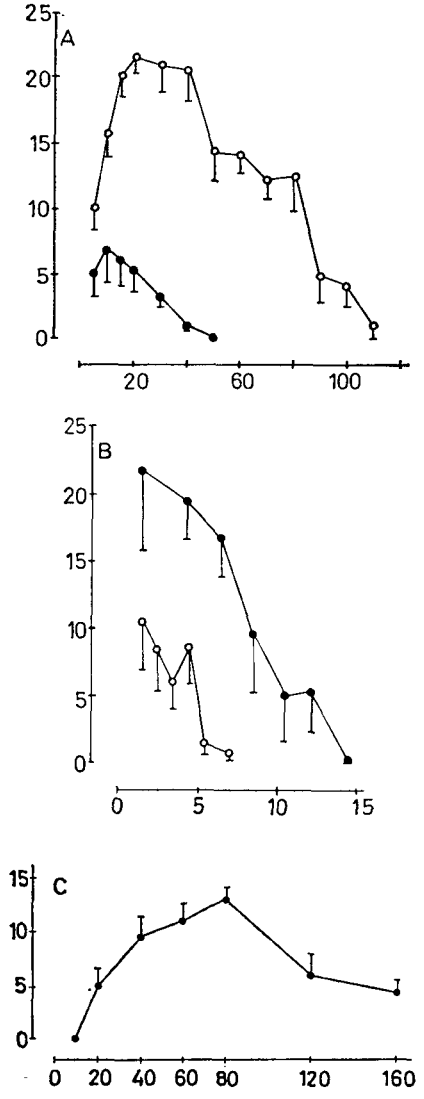


FIG. 3 A, B, C. Time course of contralateral turning behaviour after unilateral injection of muscimol (A ○—○ 25 ng ●—● 5 ng), thiomuscimol (B ○—○ 5 μg, ●—● 25 μg) and γ-acetylenic GABA (GAG) (C 40 μg) into the substantia nigra. Abscissa: min after injection. Note the difference in time scale. Ordinate: turning frequency (rev min^{-1}). Each value represents the mean ± s.e.m. of at least 4 experiments.

longer duration of muscimol. Muscimol induced turning was completely blocked in rats injected with 100 ng BMC. In addition, the stereotyped sniffing and head movements were antagonized and the rats appeared sedated. As the turning reappeared, it was followed by the typical stimulation. The injection of 0·5 μl H_2O induced only a small, non-significant

Table 3. Comparative duration of the contralateral turning induced by unilateral intranigral injection of GABA- ergic compounds into substantia nigra. The dose was selected just above the ED50 value (see Table 1 and 2).

Compound	Dose (μg)	Duration* (min)
Muscimol	0·01	75 ± 2
THIP	0·3	55 ± 8
S-(—)-5′-Methylmuscimol	1	68 ± 4
R-(+)-5′-Methylmuscimol	25	90 ± 2
RS-5′-Methylmuscimol	1	62 ± 7
Homomuscimol	3	90 ± 12
4-Methylmuscimol	15	60 ± 8
Thiomuscimol	5	7
Azamuscimol	6	46 ± 11
Isomuscimol	50	91 ± 8
Isoguvacine	3	>180
Isonipecotic acid	50	9
t-ACA	50	7
Methyl-t-ACA	10	>90
3-APS	50	120 ± 17
Methyl-GABOB	50	12 ± 3
4-Hydroxy-Butyrate	50	13 ± 3
Nipecotic acid	50	13 ± 3
Guvacine	50	6
EOS	50	13 ± 3
GAG	40	>200

* Mean ± s.e.m. of 4–6 experiments.

decrease in muscimol-induced turning. BMC 25 ng alone had no effects whereas BMC 100 ng induced a short-lasting (5–15 min) ipsilateral turning with slow frequency ranging from 2–5 rev min^{-1}. Spontaneous turning was followed by a period of ipsilateral asymmetry lasting for 20–30 min after injection. The muscimol-induced turning was also blocked by picrotoxin 100–250 ng, but was not affected by

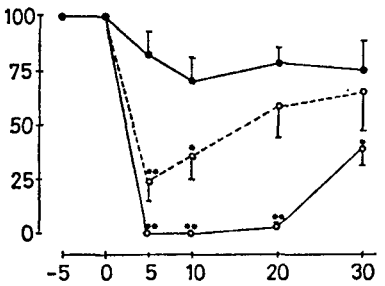


FIG. 4. Antagonism by intranigral bicuculline methochloride (BMC ○ - - - - ○ 25 ng, ○——○ 100 ng) of muscimol-induced contralateral turning after injection into the susbtantia nigra. Muscimol (25 ng in 0·5 μl) was injected 25 min before BMC (25 or 100 ng in 0·5 μl) or bidistilled water (0·5 μl, ●—●). Abscissa: min after injection of BMC or water. Ordinate: per cent of turning frequency (rev min^{-1}) obtained before BMC or water. 100% was equal to 15 ± 2 rev min^{-1}. Each value represents the mean ± s.e.m. of 4–5 experiments. * $P < 0.05$; ** $P < 0.002$ with respect to the muscimol-water group.

strychnine 1 μg, phentolamine 5–10 μg, haloperidol 2·5 μg or methysergide 2 μg, injected into the same area of SNR.

DISCUSSION

The present results showed wide ranges in potency of the compounds and thus fulfilled the criteria for a quantitative evaluation of structure activity relationships in vivo. The direct application of drugs in the vicinity of the receptors avoids transport problems across the blood-brain barrier and the immediate effect makes it less likely that turning is mediated by metabolites. Furthermore, the behavioural effect may be constant for long periods (Fig. 3, Table 1) thus making possible studies of pharmacological interactions with other drugs using each rat as its own control. The selective antagonism by BMC and the stereospecificity of the response further provide evidence that GABA-receptors are involved in the turning response (Table 1; Figs 2, 4). The difference in potency of the two optical isomers of 5′-methylmuscimol correlated closely to the comparative affinities for ^{3}H-GABA receptors (Table 1; Krogsgaard-Larsen et al 1978).

With regard to structural analogues of muscimol there was good agreement with results obtained in microelectrophoretic studies and in GABA receptor affinity binding experiments (Table 1; Krogsgaard-Larsen et al 1975; Enna & Snyder 1977; Krogsgaard-Larsen 1978; Krogsgaard-Larsen & Johnston 1978; Olsen et al 1978). Muscimol was by far the most potent GABA-agonist. Minor modifications in the muscimol molecule resulted in dramatic decreases and loss of activity (Table 1). Structure-activity consideration of muscimol and THIP have led to the hypothesis that the active conformation of GABA with respect to the postsynaptic action is the partially folded and almost planar molecule, as discussed elsewhere (Krogsgaard-Larsen et al 1977; Krogsgaard-Larsen 1978; Krogsgaard-Larsen & Johnston 1978). The only marked discrepancy within this structural class of GABA agonists was thiomuscimol, which was almost equipotent with muscimol electrophysiologically and in affinity binding (Krogsgaard-Larsen 1978) but 500-fold weaker than muscimol after injection into the substantia nigra. A plausible explanation may be that the sulphur atom in the ring structure makes this compound much more lipophilic, thus facilitating rapid diffusion away from the injection area. A rapid decrease of the amount of active substance may also be reflected in the flat slope of the dose-response curve for thiomuscimol. Alternatively, the low potency and short duration may reflect rapid metabolism. However, when potencies in different models are being compared, it must be remembered that the affinity binding studies are made on whole rat brain homogenates and micro-electrophoretic experiments on feline spinal neurons thus leaving the possibility open that species and regional differences can account for some discrepancies. It has been observed that microelectrophoretically applied muscimol is much less effective on cat cortical neurons compared with spinal neurons (Curtis et al 1971).

The long duration of isoguvacine together with its specificity as a GABA receptor agonist (Table 3; Krogsgaard-Larsen et al 1977) makes this compound very interesting as a model compound for GABA receptor interaction studies. Unfortunately, isoguvacine does not seem to cross the blood-brain barrier easily, thus prohibiting studies using systemic administration in animals (unpublished results).

t-ACA and 3-APS are potent GABA agonists structurally unrelated to muscimol (Crawford & Curtis 1964; Bowery & Brown 1974; Johnston et al 1975a; Enna & Snyder 1977; Nistri & Corradetti 1978). Both compounds and especially t-ACA were remarkably weak in this model even after extremely high doses (Table 2). t-ACA has been found to be a better substrate for GABA-transaminase than GABA itself (Beart & Johnston 1973a). Furthermore, it is a GABA uptake inhibitor which is probably cotransported into the terminals (Beart et al 1972). Both effects will most likely reduce the amount of active substance at the receptors. This is in accordance with the weak turning response after intranigral GABA (Scheel-Krüger et al 1977). Indirect support for this interpretation is that methyl-t-ACA was much more potent than t-ACA. In contrast, methylsubstitution in the same position of muscimol resulted in a marked reduction of potency. A probable explanation is that methyl-t-ACA is not a substrate for GABA-transaminase or GABA transport carrier. The weak effect of 3-APS seems more surprising since the arguments made above for t-ACA cannot be made for 3-APS which is only weak as a GABA-T substrate and as an inhibitor of GABA uptake (Beart & Johnston 1973a, b). Diffusion from the injection site is unlikely since 3-APS is completely ionized at physiological pH. However, in a recent report Nistri & Corradetti (1978) found that 3-APS had a different mechanism of action at the cellular level on frog spinal cord than GABA. Further studies are required to elucidate these differences.

The potent GABA-uptake inhibitors (−)-nipecotic acid and guvacine (Johnston et al 1975b;

1976a) both induced a short-lasting turning response (Tables 2, 3). This is probably mediated via a short-lasting release and uptake inhibition of GABA which is terminated when the uptake inhibitor is transported into the terminals (Johnston et al 1976b). Neither drug has significant affinity for postsynaptic GABA receptors in vitro (Krogsgaard-Larsen 1978; Olsen et al 1978) or significant depressant effect on single neurons (Krogsgaard-Larsen et al 1975; Lodge et al 1977).

The effect of the irreversible GABA-T inhibitor GAG (Jung et al 1977) could be clearly separated from those of the GABA agonists in the time course of turning. The agonists were immediately acting whereas the effect of GAG slowly developed during about 1 h (Fig. 2), which probably parallelled the accumulation of GABA in substantia nigra. Furthermore, the maximal effect of GAG was only approximately 50% of the turning rates obtained with GABA agonists. Whether this is a general phenomenon must await studies of more potent GABA-T inhibitors as we were unable to further increase the dose of GAG. EOS, the other GABA-T-inhibitor tested, proved to be extremely weak, in agreement with other studies (Table 2; Dray et al 1977; Tanner 1978).

The antagonist specificity of the turning response and the correlation with ^{3}H-GABA receptor affinities confirm and extend our preliminary and unexpected finding of behavioural stimulation following increased GABA receptor activity in substantia nigra (Scheel-Krüger et al 1977). This is in contrast to current hypotheses of nigral GABA functioning mainly as an inhibitory modulator of dopaminergic activity (Tarsy et al 1975). Our results may, on the other hand, suggest that the significance of the striatonigral GABA pathway alternatively could be as a mediator of behavioural stimulation on a step beyond forebrain dopaminergic activity, since these effects show similarities to dopamine receptor stimulation in striatum and/or other forebrain structures.

It must be emphasized that contralateral turning is not a sufficient criterion for classification of a drug as a GABA agonist. The neuronal organization of substantia nigra is extremely complex (for review, see Dray & Straughan 1976) and other putative neurotransmitters are able to induce turning, including substance P, noradrenaline, glycine, opiates and the glutamate analogue kainic acid (our unpublished results; McG Donaldson et al 1976; Mendez et al 1976; Di Chiara et al 1977; Iwamoto & Way 1977; James & Starr 1977; Olpe & Koella 1977). However, the contralateral turning after opiates depends on receptors situated more rostrally in substantia nigra (Iwamoto & Way 1977). Likewise the time course of action of kainic acid is different from GABA agonists in that the contralateral turning develops slowly corresponding to the neurotoxic action (Di Chiara et al 1977). In a separate investigation the specificity of muscimol-induced contralateral turning has been investigated in detail. The results indicated that only GABA antagonists specifically block muscimol (Arnt & Scheel-Krüger, to be published). In conclusion on condition that a drug in receptor binding studies has been found to be a GABA agonist we suggest that this behavioural model represents a useful and sensitive approach for studying structural requirements of GABA receptors in vivo.

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