PROCEEDINGS OF THE
British Pharmacological Society

30th–31st March, 1977

UNIVERSITY OF LIVERPOOL

COMMUNICATIONS

GABA-like properties of flurazepam and baclofen suggested by rotational behaviour following unilateral intranigral injection: a comparison with the GABA agonist muscimol

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There is much interest in the possible involvement of γ-aminobutyric acid (GABA) in mediating certain pharmacological properties of benzodiazepines (Haefely, Kulscar, Möhler, Pieri, Polc & Schaffner, 1975). Unilateral elevation of GABA levels in the zona reticulata of the rat substantia nigra (SNR) produces ipsilateral rotation under the influence of amphetamine (Dray, Fowler, Oakley, Simmonds & Tanner, 1975). A similar induction of rotational behaviour is produced by unilateral injection of the benzodiazepine chlordiazepoxide into the SNR of amphetamine-pretreated animals, suggesting an enhancement of GABA transmission by chlordiazepoxide; this effect was abolished by the GABA antagonist picrotoxin and mimicked by the GABA derivative baclofen (Waddington, 1976).

Unilateral elevation of GABA levels in SNR has recently been shown to produce contralateral rotation in the absence of treatment with dopamine agonists (Koob, Del Fiacco & Iversen, 1976); if benzodiazepines do enhance GABA transmission then their unilateral injection into SNR should similarly induce contralateral rotation without pretreatment. The effects of such injections of the potent soluble benzodiazepine flurazepam hydrochloride and baclofen were compared with those of muscimol, which has pharmacological properties suggesting GABA agonist activity (Curtis, Duggan, Felix & Johnston, 1971).

Male Sprague-Dawley rats, 150 ± 20 g, were anaesthetized with ether and given histologically confirmed unilateral stereotaxic injections of flurazepam, baclofen or muscimol into SNR in 1 µl saline; control animals received 1 µl saline. Immediately following injection animals were placed in automated rotameter bowls and rotations measured continuously for 1 hour. Control animals showed a weak ipsilateral rotation on recovery. Both flurazepam

(1–20 μg, $P<0.001$) and baclofen (5–25 ng, $P<0.001$) injections produced a vigorous dose-dependent contralateral rotation; these effects were mimicked by injections of muscimol (1 ng, $P<0.005$).

Considerable debate has centred on whether striatonigral GABA neurones are excitatory or inhibitory on nigrostriatal dopamine neurones (Dray & Straughan, 1976) whose asymmetric activity is presumed to underlay rotational behaviour; this demonstrated induction of contralateral rotation in the absence of any pretreatment suggests an excitatory process. Whether or not this is the case flurazepam produces effects identical to those produced by unilateral elevation of SNR GABA levels and SNR injections of both the GABA analogue baclofen, recently shown electrophysiologically to produce GABA-like depression of activity that is antagonized by the GABA antagonist bicuculline (Puil, Krnjevic and Werman, 1976), and the GABA agonist muscimol. These results suggest that baclofen may have some GABA agonist activity and further emphasize the GABA-like properties of benzodiazepines; whether this action is related to their clinical anxiolytic effect remains to be determined.

JLW is an MRC Scholar. Flurazepam, baclofen and muscimol were gifts from Roche Products Limited, Ciba Laboratories and Royal Dutch Shell respectively.

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Inhibition of dopaminergic activity in the extrapyramidal and limbic systems by γ-acetylenic GABA

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There is considerable evidence that γ-aminobutyric acid (GABA) acts as an inhibitory transmitter in the nigrostriatal pathway by inhibiting the ascending dopaminergic pathway at the level of the substantia nigra (Dray & Straughan, 1976). There is less evidence for a similar inhibitory role of GABA in the limbic system.

Using a new irreversible catalytic inhibitor of GABA-transaminase, γ-acetylenic GABA (GAG; RMI 71645; Jung, Lippert, Metcalf, Schechter, Böhlen & Sjoerdsma, 1977), we have examined inhibition of both the extrapyramidal and limbic dopaminergic pathways.

Three techniques were used to estimate catecholamine turnover in selected rat brain areas 4 h following GAG (100 mg/kg i.p.) administration: (a) α-methyl-*p*-tyrosine (AMPT)-induced disappearance of dopamine and noradrenaline, (b) homovanillic acid (HVA) concentrations, (c) [^{3}H]-dopamine formation following [^{3}H]-L-DOPA treatment. GAG decreased AMPT-induced dopamine disappearance in the striatum and olfactory tubercles ($P<0.005$). [^{3}H]-dopamine formation was also decreased in these regions (30 and 26% respectively, $P<0.05$) as was HVA concentration (61 and 49%, $P<0.005$). On the other hand, in the hypothalamus a small but significant ($P<0.02$) increase in dopamine turnover was found by the AMPT method and hypothalamic HVA was also increased (141%, $P<0.02$). Noradrenaline turnover was decreased in the olfactory tubercle and unchanged in the hypothalamus.

In a further series of experiments, rats received unilateral injections into the substantia nigra of 20 or 40 μg GAG in 2 μl saline or saline alone. Five hours later the rats were injected i.p. with either amphetamine (3–5 mg/kg) or apomorphine (2–6 mg/kg) and the number of rotations per minute were recorded for the following 2 hours. GAG-treated animals showed a consistent dose and time related ipsilateral turning with both amphetamine and apomorphine (e.g. 6.1 ± 0.7 turns/min after 6 mg/kg apomorphine; mean ± s.e., $n=8$), whereas saline