Rotational behaviour following inhibition of GABA metabolism unilaterally in the rat substantia nigra

On the basis of pharmacological, biochemical and electrophysiological evidence, inhibition of neuronal activity in the substantia nigra by the ipsilateral caudate nucleus is considered to be mediated by γ-aminobutyric acid (GABA) (see Tarsy, Pycock & others 1975 for refs). This inhibitory pathway may regulate the activity of the dopamine containing neurons located in the zona compacta of the substantia nigra which send projections to the striatum.

Increasing the activity of one nigro-striatal dopamine pathway in rats produces rotational behaviour towards the contralateral side. This may be achieved by electrical stimulation (Arbuthnott & Crow, 1971) or by drugs which mimic the action of dopamine. Thus after lesioning of this dopamine pathway unilaterally, the dopamine receptors in the striatum become supersensitive and systemic injection of the dopamine-receptor stimulant apomorphine causes turning away from the side of the lesion. Conversely, amphetamine which releases dopamine from nerve terminals causes turning towards the lesioned side (Ungerstedt & Arbuthnott, 1970; Ungerstedt, 1971).

We have investigated the changes in motor activity produced in rats following elevation of the endogenous GABA concentration in one substantia nigra. This was achieved by unilateral injection into the zona reticulata of ethanolamine *O*-sulphate (EOS) which is an irreversible, active-site-directed inhibitor of GABA-transaminase (GABA-T) (Fowler & John, 1972). The injection was localized to the zona reticulata since this part of the substantia nigra contains the highest concentrations of GABA (Kanazawa, Miyata & others, 1973) and glutamate decarboxylase (Fonnum, Grofova & others, 1974) and it is also the area where most of the neurons inhibited by electrical stimulation of the ipsilateral caudate nucleus are found (Dray, Gonye & Oakley, unpublished observations).

Male albino rats (OLAC, 200–250 g) were anaesthetized with halothane and unilateral intranigral injections of EOS (200 μg kg^{-1} in 1·5 μl injected over 5 min) were made with a stereotaxically positioned Hamilton syringe (A2·0–2·4; L2·0; D+2·5; König & Klippel, 1963). Animal behaviour was observed immediately after recovery from anaesthesia and then 24 h afterwards when intranigral GABA concentrations were expected to be high (Fowler, 1973). In addition, behaviour following apomorphine or amphetamine injections (4 mg kg^{-1}, i.p.) was observed. Following this, the GABA contents of the injected and the contralateral control substantia nigra from each animal were estimated using a method based on that of Lowe, Robins & Eyerman (1958). Alternatively the brains from some animals were prepared for histological verification of the injection site.

Immediately on recovery from anaesthesia most animals showed no preference in their direction of movement. Occasionally, however, EOS-injected animals exhibited contralateral postural asymmetry for periods of up to $\frac{1}{2}$ h. Twenty four hours after the EOS injection none of the animals showed postural or motor asymmetry. However after intraperitoneal injections of apomorphine or amphetamine each animal showed tight circling behaviour only towards the side of EOS injection. The rotational behaviour of each rat was monitored over a period of 2 h and one complete rotation describing a tight circle was regarded as a turn. The mean turning frequency after apomorphine was 4 turns min^{-1} and after amphetamine was 7 turns min^{-1}. No circling behaviour was seen after apomorphine or amphetamine injections in animals which had received a unilateral injection of saline into the substantia nigra 24 h previously. Animals tested with apomorphine and amphetamine 5 days after EOS injection showed ipsilateral turning, but its frequency was reduced by more than 50%

compared with the animals tested at 24 h. No turning was observed in the same animals tested 10 days after the EOS injections. Apomorphine and amphetamine caused characteristic stereotyped behaviour (e.g. sniffing) in both EOS and saline-pretreated rats, which may be attributed to general dopamine-receptor stimulation (Ungerstedt, 1971).

In all animals pretreated with EOS there was, after 24 h, a significant increase in GABA concentration in the injected substantia nigra compared with the uninjected control side (mean 173·9 s.d. 8·2%, n = 7) but there were no such differences in saline-pretreated animals (mean 99·5, s.d. 2·5%, n = 4). The absolute GABA concentration in the uninjected control substantia nigra was 11·0, s.d. 1·2 μ mol g^{-1} wet tissue.

Serial histological sectioning of the injected substantia nigra revealed minimal tissue damage and confirmed that the site of injection was within the zona reticulata.

If GABA has an inhibitory transmitter function in the substantia nigra, and if this function were to regulate the activity of dopamine-containing cells which project to the striatum, increasing the GABA concentration in one substantia nigra by inhibiting GABA-T might result in a reduction of dopamine nigro-striatal transmission on that side. This would allow the activity in the contralateral nigro-striatal pathway to predominate and to produce rotational behaviour towards the operated side. No such spontaneous activity was observed in the present experiments at a time when the GABA concentration in the EOS-injected substantia nigra had been significantly elevated and this may be attributed to a compensation by the animal for the gradual increase in GABA. The transient contralateral asymmetry seen in some animals immediately after intranigral injections of EOS might be related to the trauma of injection which increases the activity of nearby dopamine-containing neurons.

Acute injections of both apomorphine or amphetamine produced rotational behaviour towards the EOS-injected side which would indicate a predominant driving from the contralateral striatum. However, neither compound produced rotation in saline-injected animals and, therefore, we would attribute the behavioural changes after EOS pretreatment to a unilateral increase in GABA. This interpretation is further supported by the observations that the phenomenon was reversible and that its time course parallels the duration of action of EOS *in vivo* (Fowler, unpublished). It is noteworthy that when the effects of GABA are reduced in one substantia nigra, rotational behaviour away from the treated side is seen (Tarsy & others, 1975).

The present results are consistent with a role for GABA in the regulation of nigro-striatal activity, but the mechanism underlying these behavioural changes is uncertain at present. However, the effects of discrete manipulation of GABA activity on this behavioural model may provide further insight into the role of GABA in the extra-pyramidal motor system.

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REFERENCES

ARBUTHNOTT, G. W. & CROW, T. J. (1971). *Exp. Neurol.*, **30**, 484–491.

FONNUM, F., GROFOVA, L., RINVIK, E., STORM-MATHISEN, J. & WALBERG, F. (1974). *Brain Res.*, **71**, 77–92.

FOWLER, L. J. (1973). *J. Neurochem.*, **21**, 437–440.

FOWLER, L. J. & JOHN, R. A. (1972). *Biochem. J.*, **130**, 569–573.

KANAZAWA, I., MIYATA, Y., TOYOKURA, Y. & OTSUKA, M. (1973). *Brain Res.*, **51**, 363–365.

KÖNIG, J. F. R. & KLIPPEL, R. A. (1963). *The rat brain: a stereo taxic atlas of the forebrain and lower parts of the brain stem.* Williams & Wilkins: Baltimore.

LOWE, I. P., ROBINS, E. & EYERMAN, G. S. (1958). *J. Neurochem.*, **3**, 8–18.

TARSY, D., PYCOCK, C., MELDRUM, B. & MARSDEN, C. D. (1975). *Brain Res.*, **89**, 160–165.

UNDERSTEDT, U. (1971). *Acta physiol scand.*, **82**, suppl. 367, 69–93.

UNGERSTEDT, U. & ARBUTHNOTT, G. W. (1970). *Brain Res.*, **24**, 485–493.

Prevention of paracetamol-induced liver damage in mice with glutathione

Large doses of paracetamol can produce fatal liver necrosis in animals (Boyd & Bereczky, 1966) and in man (Prescott, Wright & others, 1971). Recent mechanistic studies suggest that the hepatotoxicity is mediated through the formation of an active metabolite which covalently binds to liver macromolecules (Mitchell, Jollow & others, 1973a; Jollow, Mitchell & others, 1973). Furthermore, the liver damage due to paracetamol is related to depletion of the hepatic glutathione (Mitchell, Jollow & others, 1973b). However, there appear to be no reports in the literature about the effect of glutathione administration on paracetamol hepatotoxicity, although precursors such as cysteine (Mitchell & others, 1973b) and the related compound cysteamine (Prescott, Swainson & others, 1974) are protective.

In an investigation on the use of liposomes as carriers of potential protective agents against drug-induced liver necrosis (Strolin Benedetti, Louis, Malnoë, Schneider, Smith, Lam & Kreber, unpublished results) it was found that glutathione injected intravenously could largely protect mice against the heptotoxic effect of large doses of paracetamol and these findings are now reported.

Paracetamol 18·9 mg ml in 0·9% saline was injected intraperitoneally into groups of at least 10 male mice of Swiss strain, 25 $\pm$ 3 g, which had been fasted overnight, at 500 mg kg^{-1}. This dose regularly produced liver damage, the extent of which varied considerably from animal to animal and from experiment to experiment. Glutathione, in 0·9% saline, was injected intravenously into the tail vein at doses from 32–800 mg kg^{-1} as follows: (a) as a single dose given at either 15, 45, 105 or 180 min after the dose of paracetamol; (b) as four equal divided doses given at 15, 45, 75 and 105 min before and (c) after the paracetamol.

Paracetamol induced liver damage was assessed by measurement of plasma glutamic - oxaloacetic transaminase (GOT) and glutamic-pyruvate transaminase (GPT) and by histopathological examination. Twenty-four hours after drug treatment dead mice were counted and the survivors killed by cutting the carotid artery under slight ether anaesthesia. Blood samples were taken from each animal and the serum transaminases estimated according to Reitman & Frankel (1957) and the results expressed in terms of international units $litre^{-1}$. The livers were removed and examined for