Br. J. Pharmac. (1977), **60**, 29–34

RESPONSES OF MOTONEURONES TO ELECTROPHORETICALLY APPLIED DOPAMINE

S. BARASI & M.H.T. ROBERTS
Physiology Department, University College, PO Box 78, Cardiff CF1 1XL

1 The effect of electrophoretically applied dopamine upon motoneurone excitability has been investigated. Field potentials originating from antidromically activated motoneurones were recorded from the ventral horn of the rat lumbar spinal cord.

2 Field potentials showed an increase in amplitude following electrophoretic application of dopamine. Dopamine was shown to be less potent than noradrenaline and 5-hydroxytryptamine in producing these changes.

3 Measurement of the transport number of dopamine suggests that the relatively low potency of dopamine cannot be attributed to differences in ionic mobilities between the amines.

4 Electrophoretic application of α-flupenthixol was shown to discriminate between dopamine and 5-hydroxytryptamine responses. Dopamine responses were profoundly reduced.

5 Electrophoretically applied α-flupenthixol also discriminated between dopamine and noradrenaline. Noradrenaline responses were consistently potentiated by α-flupenthixol. The possibility is discussed that dopamine may not merely be a precursor for noradrenaline in the rat spinal cord.

Introduction

Using the Falck-Hillarp technique, Dahlström & Fuxe (1965) reported the existence of descending monoaminergic pathways in the spinal cord. They observed noradrenaline and 5-hydroxytryptamine containing terminals in close proximity to lumbar motoneurones. Subsequent experiments have provided good evidence to suggest a neurotransmitter role for the amines in the spinal cord. In contrast, the relatively small quantities of dopamine present in the spinal cord have been regarded as having an entirely precursor role to noradrenaline. Recently, however, Commissiong & Sedgewick (1974) and Magnusson (1973) have suggested that dopamine is present in the spinal cord in amounts which indicate that the amine may have a neurotransmitter role.

The present study was carried out to determine whether dopamine applied locally by microelectrophoresis has any pharmacological effects on spinal motoneurones and whether these effects can be blocked by a drug considered to be a specific antagonist of responses to dopamine.

Methods

Experiments were performed on Wistar rats of either sex anaesthetized with fluothane. Blood pressure and ECG were routinely monitored. Laminectomy was performed between lumbar and sacral segments and ventral roots were cut about 1 cm from the cord.

Drugs were applied by microelectrophoresis from five-barrelled micropipettes. Solutions were made up as follows: 5-hydroxytryptamine bimaleate (Koch-Light) 0.2 M, pH 3.0, noradrenaline hydrochloride (Koch-Light) 0.2 M, pH 3.5, the diacetate salt of α-flupenthixol (Lundbeck) 0.2 M, pH 3.5 and dopamine hydrochloride (Sigma) 0.2 M, pH 3.5.

Extracellular field potential responses of lumbar motoneurones were evoked by regular antidromic stimulation of the central end of transected ventral roots and recorded through a sodium chloride filled barrel of the micropipette. Square wave pulses of 0.5 ms duration of varying intensity were used to deliver stimulation to ventral roots. Field potentials originating from motoneurones were displayed on an oscilloscope and their amplitude recorded on a pen writing polygraph. Field potentials changed in amplitude when drugs were applied. The change of the field potential amplitude and the period for which the change persisted was defined as the response to the electrophoretically applied drug. Quantification of drug effects involved measuring the area enclosed by the response. Response areas were measured with a planimeter. An example of the procedure is shown in Figure 1d (see also legend).

Further details of the experimental procedures have been published elsewhere (Barasi & Roberts, 1974).

Determination of transport numbers

A series of *in vitro* experiments was performed to investigate the transport number of dopamine and noradrenaline. Transport numbers were obtained by the use of five-barrelled micropipettes similar to those used in the *in vivo* experiments.

Freeze-dried (±)-noradrenaline [carbinol-^{14}C]-(±)-bitartrate (specific activity 35 mCi/mmol) was mixed with non-radioactive noradrenaline to provide a final solution of 0.2 M. A solution of similar molarity was made from [ethylamine-2-^{14}C]-dopamine hydrochloride and non-radioactive dopamine hydrochloride. The pH of the final solutions was 3.5.

The effects of 25, 50, 100 and 150 nA ejecting current was studied in all pipettes. On each occasion the pipette tip was lowered into a perspex vial containing 1 ml of 0.165 M NaCl. An electrophoretic current was applied for 10 min following which the 1 ml sample was transferred to a counting vial containing 10 ml of scintillator (Nuclear Enterprises NE 260). This was repeated three times for each current. Disintegrations originating from each radioactive sample were counted during a 10 min period in a Nuclear Enterprises liquid scintillation counter.

Results

Motoneurone field potentials

The amplitude of antidromically evoked motoneurone field potentials depended upon the position of the micropipette and the intensity of the ventral root stimulation. Field potentials were generally first recorded at a depth of about 1000 μm from the dorsal surface of the cord. As the micropipette penetrated further towards the ventral horn the amplitude of the recorded potential increased frequently to about 0.7 mV. Increasing the intensity of ventral root stimulation (to a maximum of about 0.3 V) brought about a concomitant increase in field potential amplitude. Stimulation voltage was adjusted at a level twice that required to give a just maximum field potential. Figure 1a illustrates a typical antidromically evoked field potential recorded from the rat spinal cord.

Although no obvious relationship was noted between responsiveness of the field potential to locally applied drugs and location (depth) of the micropipette, we did find that the larger field potentials recorded between about 1500 and 2000 μm were influenced less by drugs.

Providing the blood pressure remained constant, field potential amplitude fluctuated little. However, almost invariably, a fall in blood pressure resulted in an increase in field potential amplitude and some preparations were abandoned for this reason.

Response to electrophoretically applied dopamine

Dopamine was applied electrophoretically whilst recording antidromically evoked motoneurone field potentials in 91 positions in 21 animals. Currents used to eject dopamine varied between 75 and 150 nA.

An example of the increase in motoneurone field potential in response to the application of dopamine is shown in Figure 1b. The increase in field potential amplitude was dependent upon the intensity of the electrophoretic current and duration of the application. The extent of the increase varied between about 30% and 100%. On 45 occasions (50%) the field potential was increased in amplitude whilst on only 2 occasions was a small reduction in amplitude recorded. Dopamine was ineffective on 45 occasions (50%) in changing field potential amplitude. In 18 of these studies 5-hydroxytryptamine or noradrenaline evoked marked changes in the field potential.

Figure 1c and d shows how the response to the same amine varied from one recording position to another. The more usual response was a graded increase in amplitude (Figure 1d), the amplitude of the field potential showed an approximately regular increase during the application of the amine. In some recording positions discrete step-like increases in amplitude were recorded. Figure 1c illustrates a study in which this effect was observed, and it can be seen that both 5-hydroxytryptamine and dopamine increased the field potential amplitude incrementally. In those positions in the spinal cord where noradrenaline and dopamine were effective and were ejected with similar currents for similar periods of time, noradrenaline invariably produced the larger response. In 10 such studies the average noradrenaline response was 2.2 ± 0.2 times greater in area than the dopamine response.

Antagonism studies

Discrimination betwen dopamine and 5-hydroxytryptamine. 5-Hydroxytryptamine and dopamine were ejected with electrophoretic currents whose intensity and duration were adjusted to give comparable response areas; 25–50 nA of α-flupenthixol was then applied electrophoretically for 10–15 minutes. The results of one study is shown in Figure 2. Each filled symbol on the graph (Figure 2c) represents the area enclosed by a single response to 5-hydroxytryptamine or dopamine. Two excerpts from the record are shown. Figure 2a illustrates responses to 5-hydroxytryptamine and dopamine before the application of α-flupenthixol whilst Figure 2b illustrates the size of the amine responses following application of the antagonist. The open circles on the graph show that the amplitude of the individual field potentials measured just before application of either agonist was uninfluenced by α-flupenthixol. The antagonist did, however, reduce dopamine responses to 25% of pre-

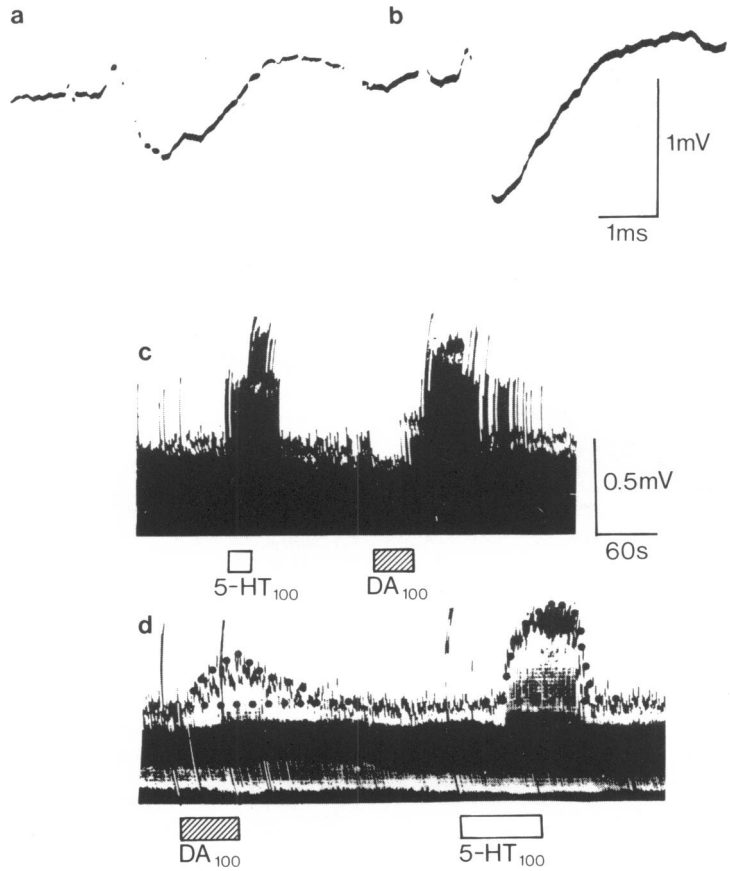

Figure 1 Responses of motoneurone field potentials to electrophoretically applied amines. (a) The negative-going motoneurone field potential recorded from the ventral horn in response to antidromic stimulation of ventral roots. Field potentials were evoked at the rate of between 12 and 20 per minute. (b) Increase in amplitude of the field potential in response to the electrophoretic application of 100 nA of dopamine (DA). (c) Step-like increase in field potential amplitude in response to electrophoretically applied amine. Each line represents one motoneurone field potential. Changes in the height of the lines reflect changes in amplitude of the field potential. Bars and numbers beneath the record indicate the duration and intensity (nA) (respectively) of electrophoretic currents (d) Graded response to electrophoretically applied amines. Responses were quantified by measuring the area enclosed by the dotted line.

antagonist levels without affecting 5-hydroxytryptamine responses.

Recovery from the maximal dopamine blockade began shortly after termination of the α-flupenthixol application but was incomplete 110 min later. α-Flupenthixol has shown selective blockade of this nature on four occasions. In 4 other studies, however, the antagonist blocked responses to both 5-hydroxytryptamine and dopamine, although on no occasion was the 5-hydroxytryptamine response preferentially blocked. High doses of α-flupenthixol (>75 nA for 5 min or more) decreased the amplitude of the motoneurone field potential.

Discrimination between dopamine and noradrenaline. Electrophoretically applied dopamine has a relatively weak effect on motoneurone field potentials, and obtaining alternate reproducible dopamine and noradrenaline responses proved rather difficult. Figure 3c shows a study in which α-flupenthixol successfully discriminated between the two amines. Following two control applications of each amine, 50 nA of α-flupenthixol was applied for 7 minutes. The dopamine response was reduced to 30% of pre-antagonist level but showed complete recovery. The effect of α-flupenthixol on noradrenaline responses was quite different: there was

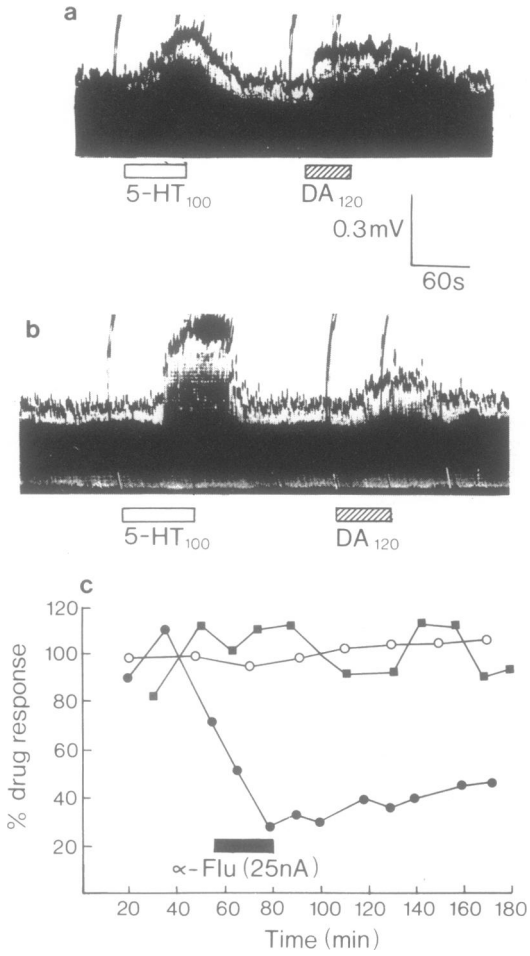

Figure 2 Effect of α-flupenthixol on 5-hydroxytryptamine and dopamine responses. (a) Excerpts from the records illustrating the responses of motoneurone field potentials to 5-hydroxytryptamine (5-HT) and dopamine (DA) (b) Responses to 5-hydroxytryptamine and dopamine following the application of α-flupenthixol. The amplitude and time calibration marks are applicable to both excerpts. (c) Graph representing the entire study of which (a) and (b) were excerpts. α-Flupenthixol (α-Flu) has discriminated between the two amine responses. The 100% response level corresponds to the mean amine response before application of α-flupenthixol. 5-Hydroxytryptamine responses (■); dopamine responses (●); individual field potential amplitude evoked in the absence of locally applied amine (○).

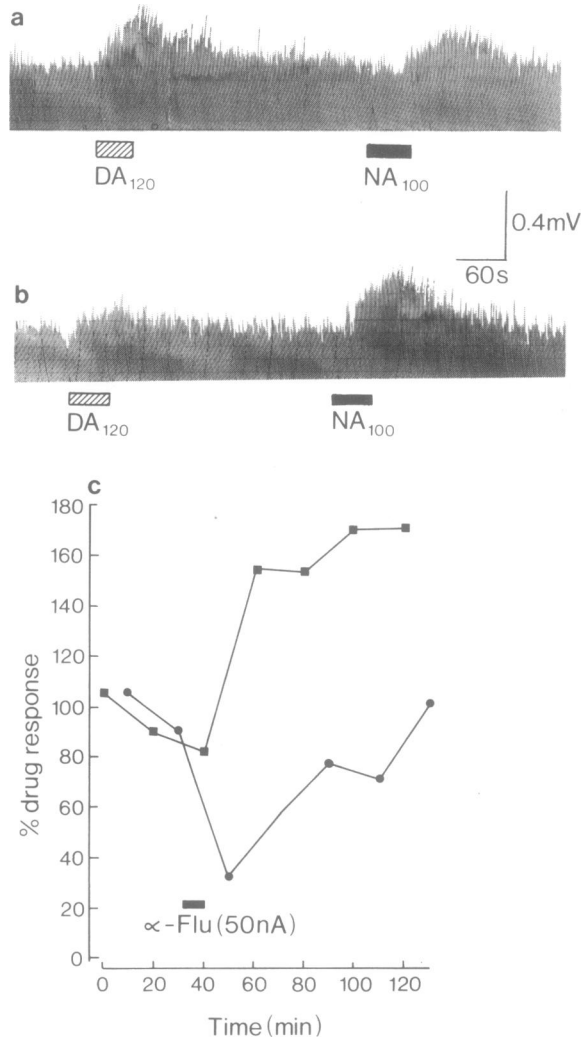

Figure 3 Effect of α-flupenthixol on dopamine and noradrenaline responses. (a) Excerpts from records illustrating responses to dopamine (DA) and noradrenaline (NA) in the absence of antagonist. Calibration marks refer to both (a) and (b). (b) Responses to dopamine and noradrenaline following the application of α-flupenthixol. (c) Graph illustrating the complete study. The dopamine antagonist has reduced the dopamine response and potentiated the noradrenaline response. The 100% response level corresponds to the mean amine response before application of α-flupenthixol (α-Flu). Noradrenaline responses (■); dopamine responses (●).

a clear potentiation which in this study showed no recovery. Figure 3a and b illustrates selected responses from the study.

Potentiation of the noradrenaline response following α-flupenthixol was observed on 4 occasions and the antagonist discriminated between the 2 amines in 5 studies.

In several studies spontaneous firing was recorded following application of the antagonist. The firing was usually associated with a reduction in the control field

potential amplitude, that is on those occasions when excess antagonist was applied.

Transport number determinations

The average transport number obtained from 4 micropipettes for noradrenaline was 0.155 ± 0.002 (mean $\pm$ s.e. mean) and from 5 micropipettes for dopamine was 0.392 ± 0.001. These values represent the average of 15 release readings from each pipette. The resting release (corresponding to 0 nA) was subtracted from the ejection release before the transport number was calculated. The mean resting release from all pipettes was 1.3 ± 0.2 pmol/min per barrel.

Discussion

The increase in field potential following dopamine application probably reflects an increase in the excitability of motoneurones (Barasi & Roberts, 1974). The ability of α-flupenthixol to discriminate between responses to 5-hydroxytryptamine and dopamine and noradrenaline and dopamine suggests that dopamine responses do not result from an action upon a generalized amine receptor.

The specificity of monoamine receptor blocking drugs varies markedly. However, it seems likely that the thioxanthene derivative α-flupenthixol is a relatively specific dopamine antagonist. A comparison of the relative potencies of a range of antipsychotic drugs in inhibiting dopamine-stimulated adenylate cyclase (Iversen, 1975), suggests that α-flupenthixol is the most potent of the drugs currently available.

Two studies have been described (Ben-Ari & Kelly, 1974; House & Ginsborg, 1976) which indicate that dopamine effects can be blocked by α-flupenthixol. The present experiments compare the effect of α-flupenthixol on responses to dopamine, noradrenaline and 5-hydroxytryptamine. The results suggest that spinal motoneurones or adjacent units are sensitive to locally applied dopamine and that the receptors involved are blocked by an antagonist believed to be specific for dopamine.

α-Flupenthixol potentiated responses of motoneurones to noradrenaline. This unexpected observation seems to support the suggestion of Brown & Makman (1972) and Kebabian, Petzgold & Greengard (1972) that dopamine receptors are pharmacologically distinct from β-adrenoceptors. The potentiation of noradrenaline responses by α-flupenthixol has not been reported previously and no explanation of this effect is presently justified.

Values for the transport number of noradrenaline from bitartrate solutions are comparable to previous determinations (Bradshaw, Roberts & Szabadi, 1973; Candy, Boakes, Key & Worton, 1974). However, the transport number for dopamine from hydrochloride solutions was found to be more than twice that recorded for noradrenaline. Consequently the differences in the size of the noradrenaline and dopamine responses cannot be attributed to differences in mobilities of the two ions and suggests that dopamine is much less potent on lumbar motoneurones than noradrenaline.

It has been suggested (Candy *et al.*, 1974) that electrophoretically applied drugs may, under diffusional influences, travel many hundreds of microns from the micropipette tip. Under these circumstances, it is possible that the present effects may have been mediated indirectly via interneurones close to the motoneurones. However, it was only rarely that recordings of spontaneous activity ever accompanied the motoneurone field potential. It thus seems unlikely that the effects of electrophoretically applied amine were consistently being mediated via interneurones.

The difference in response characteristics between one recording site and another may be associated with the spatial relationship between the recording electrode and the motoneurone. The step-wise increase in field-potential amplitude illustrated in Figure 1c may reflect the lowering of the threshold of two motoneurones located close to the recording microelectrode. In the absence of amine the motoneurones would remain univaded by the antidromic action potential. Following the application of amine the threshold of the motoneurones was perhaps lowered and the antidromic impulse invaded the cells. The more regular shape of drug responses illustrated in Figure 1d probably results from similar changes in excitability occurring in motoneurones at some distance from the recording microelectrode.

There is conflicting evidence concerning the existence of dopamine within the spinal cord. Magnusson & Rosengren (1963), Atack (1963), Anton & Sayer (1964) and Laverty & Sharman (1965), all reported dopamine concentrations within the range 10–20 ng/gram. McGeer & McGeer (1962), however, found dopamine in concentrations of about 1000 ng/g in rabbit and rat cord. More recently Commissiong & Sedgewick (1974), initially investigating dopamine concentrations in the spinal cord of rat, reported concentrations of 130 ng/gram. Subsequently in human spinal cord the same authors found similar (up to 190 ng/g) concentrations (Commissiong & Sedgewick, 1975).

Magnusson (1973) monitored changes in concentration of noradrenaline and dopamine in rat spinal cord following transection and noted a different time course for the disappearance of the two amines. This evidence may suggest that the dopamine existing in the spinal cord is not inevitably associated with noradrenaline.

Further evidence supporting the existence of a descending dopamine pathway comes from experiments in which electrical stimulation of the substantia

nigra changed spinal monosynaptic reflex activity (York, 1972). These effects were not mediated via the supraspinal structures but were consistently blocked by agents thought to have dopamine blocking activity (chlorpromazine and haloperidol).

The authors wish to thank the Parkinson's Disease Society for financial assistance in supporting the present work. Thanks are also extended to H. Lundbeck & Co. A/S who generously donated α-flupenthixol.

References

ANTON, A.H. & SAYER, D.F. (1964). The distribution of dopamine and dopa in various animals and a method for their determination in diverse biological material. *J. Pharmac. exp. Ther.*, **145**, 326–336.

ATACK, C.V. (1963). The determination of dopamine by a modification of the dihydroxyindole fluorimetric assay. *Br. J. Pharmac. Chemother.*, **38**, 699–714.

BARASI, S. & ROBERTS, M.H.T. (1974). The modification of lumbar motoneurone excitability by stimulation of a putative 5-hydroxytryptamine pathway. *Br. J. Pharmac.*, **52**, 339–348.

BEN-ARI, Y. & KELLY, J. (1974). Iontophoretic and intravenous effects of the neuroleptic α-flupenthixol on dopamine evoked inhibition. *J. Physiol., Lond.*, **242**, 66–67P.

BRADSHAW, C.M.O., ROBERTS, M.H.T. & SZABADI, E. (1973). Kinetics of the release of noradrenaline from micropipettes: interaction between ejecting and retaining currents. *Br. J. Pharmac.*, **49**, 667–677.

BROWN, J.H. & MAKMANN, J. (1972). Stimulation by dopamine of adenylate cyclase in retinal homogenates and of adenosine-3′,5′-cyclic monophosphate formation in intact retina. *Proc. Natl Acad. Sci. U.S.A.*, **69**, 539–543.

CANDY, J.M., BOAKES, R.J., KEY, B.J. & WORTON, E. (1974). Correlation of the release of amines and antagonists with their effects. *Neuropharmac.*, **13**, 423–430.

COMMISSIONG, J.W. & SEDGEWICK, E.M. (1974). On the presence of dopamine in the mammalian spinal cord. *Br. J. Pharmac.*, **51**, 118–119P.

COMMISSIONG, J.W. & SEDGEWICK, E.M. (1975). Dopamine and noradrenaline in the human spinal cord. *Lancet*, **i**, 347.

DAHLSTRÖM, A. & FUXE, K. (1965). Evidence for the existence of monoamine neurons in the central nervous system. *Acta physiol. scand.*, **64**, supp. 247.

HOUSE, C.R. & GINSBORG, B.L. (1976). Actions of a dopaminergic analogue and a neuroleptic at a neuroglandular synapse. *Nature, Lond.*, **261**, 332–333.

IVERSEN, L.L. (1975). Dopamine receptors in the brain. *Science*, **188**, 1084–1089.

KEBABIAN, J.W., PETZGOLD, G. & GREENGARD, P. (1972). Dopamine-sensitive adenylate cyclase in caudate nucleus of rat brain and its similarity to the 'Dopamine Receptor'. *Proc. Natl Acad. Sci. U.S.A.*, **69**, 2145–2149.

LAVERTY, R. & SHARMAN, D.F. (1965). The estimation of small quantities of 3,4-dihydroxyphenylethylamine in tissues. *Br. J. Pharmac. Chemother.*, **24**, 538–548.

MAGNUSSON, T. (1973). Effect of chronic transection on dopamine, noradrenaline and 5-hydroxytryptamine in the rat spinal cord. *Naunyn-Schmiedebergs Arch. Pharmac.*, **278**, 13–22.

MAGNUSSON, T. & ROSENGREN, E. (1963). Catecholamines of the spinal cord normally and after transection. *Experientia*, **19**, 229–230.

McGEER, E.G. & McGEER, P.L. (1962). Catecholamine content of the spinal cord. *Canad. J. Biochem. Physiol.*, **40**, 1141–1151.

YORK, D.H. (1972). Alterations in spinal monosynaptic reflex produced by stimulation of the substantia nigra. In *Corticothalamic Projections and Sensorimotor Activities*, ed. Frigyesi, T., Rinvik, E. & Yahr, M. pp. 445–447. New York: Raven Press.

(Received July 12, 1976. Revised December 16, 1976.)