for 18 h at room temperature. The gel was then washed with 0.1 M $NaHCO_3$ followed by distilled water and was then equilibrated with 1 mM phosphate buffer pH 7.8. Sonicated soluble rat liver MAO was then applied to the gel and washed through with buffer. The MAO enzyme was eluted in the unbound fraction and was 5.6 times purer than the starting material with a 100% recovery of the enzyme. The bound protein was eluted from the organomercurial-sepharose column with 50 mM cysteine in 0.2 M phosphate buffer at pH 7.4 containing 0.01 M EDTA.

A tranylcypramine-sepharose column was prepared by attaching tranylcypramine to succinyl aminohexane sepharose. Soluble MAO applied to this column was not bound nor was there an increase in the specific activity of the eluate from the column.

A sepharose column was also prepared by attaching 1-meta-aminophenyl-2-cyclo-propyl-aminoethanol (AB-15), to succinyl amino hexane-sepharose. Soluble MAO was applied to this column and 10% of the enzyme together with 15% of the protein was bound to the column. Elution of the column with 0.2 M phosphate buffer, pH 7.6, recovered only 1% of the enzyme, but this fraction had a 40 fold purification over the original material.

The results suggest that affinity chromatographic techniques may provide a suitable procedure for preliminary purification of MAO.

References

CAUTRECASAS, P., WILCHEK, M. & ANFINSEN, C.B. (1968). Selective enzyme purification by affinity chromatography. *Proc. natn. Acad. Sci.*, **61**, 636-643.

ORELAND, L. (1971). Purification and properties of pig liver mitochrondrial monoamine oxidase. *Arch. Biochem. Biophys.*, **146**, 410-421.

TIPTON, K.F. (1968). The purification of pig brain mitochondrial monoamine oxidase. *Europ. J. Biochem.*, **4**, 103-107.

WILCHEK, M. & GORECKI, M. (1969). Affinity chromatography of bovine pancreatic ribonuclease A. *Europ. J. Biochem.*, **11**, 491-494.

YOUDIM, M.B.H. & SOURKES, T.L. (1966). Properties of purified, soluble monoamine oxidase. *Canad. J. Biochem. Physiol.*, **44**, 1397-1400.

Behavioural and biochemical evidence for cerebral dopamine receptor blockade by metoclopramide in rodents

P. JENNER, C.D. MARSDEN & E. PERINGER

University Department of Neurology, Institute of Psychiatry, and King's College Hospital Medical School, Denmark Hill, London SE5 8AF U.K.

Metoclopramide, a widely used anti-emetic agent, causes dyskinesias in a small percentage of patients (Borenstein & Bles, 1965; Casteels-van Daele, Jaeken, van der Schueren, Zimmerman & van der Bon, 1970); like other neuroleptic agents which cause similar dyskinesias, it may block cerebral dopamine receptors (Costall & Naylor, 1973; Janssen, Niemegeers, Schellekens & Lenaerts, 1967). These behavioural and biochemical studies described here indicate that metoclopramide is a cerebral dopamine receptor antagonist.

Metoclopramide administered i.p. 30 min before apomorphine (2 mg/kg) produced a dose-dependent inhibition of stereotopy (ED_{50} 1.5 mg/kg). The reversal of reserpine-induced suppression of locomotor activity by apomorphine (2 mg/kg) was significantly decreased by prior administration of metoclopramide (ED_{50} 17 mg/kg). Metoclopramide also antagonized the effect of apomorphine or amphetamine in producing turning behaviour in mice with unilateral lesions of the nigrostriatal pathway (ED_{50} 5.0 and 4.0 mg/kg respectively). Metoclopramide resembled pimozide in all these respects and appeared to be a relatively potent antagonist of striatal dopamine receptors in these behavioural models (Dolphin, Jenner, Marsden, Pycock & Tarsy, 1975).

Pretreatment of male mice (20-30 g) with metoclopramide (50 mg/kg i.p.) caused a time-dependent increase in homovanillic acid (HVA) in whole brain, which was maximal (740%) $1\frac{1}{2}$ h after injection and lasted some 5 hours. Metoclopramide (50 mg/kg) increased HVA concentrations to the same extent in the corpus striatum (450%) and the mesolimbic area (590%) (a slice containing corpora amygdala, nucleus accumbens and olfactory tubercle). On the other hand no change in whole brain concentrations of dopamine, noradrenaline (NA), 4-hydroxy-3-methoxyphenylglycolsulphate (MOPEG-SO4), 5-hydroxytryptamine (5HT), or 5-hydroxy-indoleacetic acid were observed after metoclopramide treatment. The biochemical results suggest that metoclopramide blocks cerebral dopamine receptors in both striatum and mesolimbic areas,

thereby increasing dopamine turnover. On the other hand the noradrenaline and 5-hydroxytryptamine systems do not seem to be influenced. (Since this work was completed Ahtee (1975) has also independently reported that metoclopramide causes a rise in whole brain HVA.)

It remains to be discovered why metoclopramide given to parkinsonian patients does not increase the severity of the parkinsonian syndrome, and does not affect the intensity of L-DOPA induced dyskinesias (Tarsy, Parkes & Marsden, 1975). It is also surprising that metoclopramide has little, if any, antipsychotic activity (Borenstein & Bles, 1965) in view of the current suggestion that this property is associated with the capacity to block cerebral dopamine receptors particularly those in the mesolimbic area (Van Rossum, Janssen, Boissier, Julou, Loew, Moller Nielsen, Munkrad, Randrup, Stille & Tedeschi, 1970; Costall & Naylor, 1973).

References

AHTEE, L. (1975). The effect of drugs on metoclopramide-induced catalepsy and increase in striatal homovanillic acid content. *Br. J. Pharmac.* In press.

BORENSTEIN, B. & BLES, G. (1965). Effets cliniques et électroencéphalographiques du métoclopramide en psychiatrie. *Thérapie,* **20**, 975-995.

CASTEELS-VAN DAELE, M., JAEKEN, J., VAN DER SCHUEREN, P., ZIMMERMAN, A. & VAN DER BON, P. (1970). Dystonic reactions in children caused by metoclopramide. *Arch. Dis. Child.*, **45**, 130-133.

COSTALL, B. & NAYLOR, R.J. (1973). Is there a relationship between the involvement of extrapyramidal and mesolimbic brain areas with the cataleptic action of neuroleptic agents and their clinical antipsychotic effect? *Psychopharmacologia,* **32**, 161-170.

DOLPHIN, A., JENNER, P., MARSDEN, C.D., PYCOCK, C. & TARSY, D. (1975). Pharmacological evidence for cerebral dopamine receptor blockade by metoclopramide in rodents. *Psychopharmacologia,* in press.

JANSSEN, P.A.J., NIEMEGEERS, C.J.E., SCHELLEKENS, K.H.L. & LENAERTS, F.M. (1967). Is it possible to predict the clinical effects of neuroleptic drugs (major tranquilisers) from animal data? *Arzneimittel-Forsch.*, **17**, 841-854.

TARSY, D., PARKES, J.D. & MARSDEN, C.D. (1975). Metoclopramide and pimozide in Parkinson's disease and levodopa induced dyskinesias. *J. Neurol. Neurosurg. Psychiat.* Submitted.

VAN ROSSUM, J.M., JANSSEN, P.A.J., BOISSIER, J.R., JULOU, L., LOEW, D.M., MOLLER NIELSEN, I., MUNKRAD, I., RANDRUP, A., STILLE, G. & TEDESCHI, D.H. (1970). *Pharmacology in "Modern Problems of Pharmacopsychiatry, The Neuroleptics",* eds. Bobon, D.P., Janssen, P.A.J. & Bobon, J. 5, 23-70, Karger, Basel.

Measurement of metabolism of resting and exercising human skeletal muscle *in situ.*

R. GREENWOOD, H. LLOYD, R.F. MOTTRAM & G. ROBERTS (introduced by V.R. PICKLES)

Department of Medicine, Welsh National School of Medicine and Department of Physiology, University College, Cardiff

Forearm blood flow is measured by the venous occlusion plethysmographic method and the flow through the muscle determined from the measured total forearm flow (Cooper, Edholm & Mottram, 1955). By catheterization of deep forearm veins from the antecubital fossa it is possible to obtain samples that are predominantly muscle effluent blood (Coles, Cooper, Mottram & Occleshaw, 1958). The simultaneous sampling of arterial blood enables arterio-venous differences across the muscles to be determined of various substances of metabolic importance. A.-V. differences multiplied by blood flow rates give the metabolic turn-over rates for these substances. Use of an isometric hand-grip ergometer, with hand grips of 5 or 10% of subjects' maximal power, allows the blood flow and sampling techniques to be performed while the muscle is exercising under controlled conditions as well as at rest (Baker & Mottram, 1973).

We are developing these methods to study the actions of substances, both naturally occurring hormones and pharmacological agents, believed to affect the tissue's metabolic activity.

References

BAKER, P.G.B. & MOTTRAM, R.F. (1973). Metabolism of excercising and resting muscle, in the post-prandial and fasting states. *Clin. Sci.*, **44**, 479-491.

COLES, D.R., COOPER, K.E., MOTTRAM, R.F. & OCCLESHAW, J.V. (1958). The source of blood samples withdrawn from deep forearm veins via catheters passed upstream from the median cubital vein. *J. Physiol.*, **142**, 323-328.

COOPER, K.E., EDHOLM, O.G. & MOTTRAM, R.F. (1955). The blood flow in skin and muscle of the human forearm. *J. Physiol.*, **128**, 258-267.