when present in the perfusion solution caused a potentiation of the responses to NA above the control values. The control ED_{50} response to NA was potentiated by 60% in the presence of imipramine and by 50% and 40% respectively in the presence of amitriptyline and clomipramine (1×10^{-6} M). At a perfusion concentration of 1×10^{-8} M, however, the ED_{50} NA response was potentiated by 30% in the presence of clomipramine, by 20% in the presence of imipramine and by 10% when amitriptyline was present. Each of the three drugs inhibited NA uptake when compared to control values in the absence of any drug. At a concentration of 1×10^{-8} M clomipramine inhibited NA uptake by 30% while amitriptyline and imipramine inhibited NA uptake by 15% and 20% respectively, compared to control values. During perfusion with imipramine (1×10^{-6} M) NA uptake was inhibited by 80% while in the presence of clomipramine and amitriptyline respectively, NA uptake was depressed by 55% and 65% respectively.

These results suggest that all three tricyclic depressants are potent inhibitors of NA uptake. Clomipramine is the most potent inhibitor of NA uptake at a perfusion concentration of 1×10^{-8} M, while imipramine is the most potent inhibitor of NA uptake at a concentration of 1×10^{-6} M. There was a direct correlation between the effects of each drug on NA uptake and the effects on arterial responsiveness to NA. The differences in potencies between clomipramine and the other tricyclic compounds at different perfusion concentrations might explain the greater effectiveness of clomipramine in some disorders.

References

GEORGE, A.J. & LEACH, G.D.H. (1973). The effects of changes in ionic environment and modification of adrenergic function on the vascular responses to sympathomimetic amines. *J. Pharm. Pharmac.*, **25**, 521–529.

GEORGE, A.J. & LEACH, G.D.H. (1975). The involvement of Ca^{2+} and Mg^{2+} in the spontaneous and drug induced release of ^{3}H-noradrenaline from mesenteric arteries. *Biochem. Pharmac.*, **24**, 737–741.

IVERSEN, L.L. (1963). The uptake of noradrenaline by the isolated perfused rat heart. *Br. J. Pharmacol.*, **21**, 523–527.

IVERSEN, L.L. (1967). The uptake and storage of noradrenaline. London: Cambridge University Press.

RACK, P.H. (1973). A comparative trial of oral clomipramine against imipramine. *J. Int. Med. Res.*, **1**, 332–337.

Stereoselective uptake of L-homocysteate by rat brain slices

D.W.G. COX & J.C. WATKINS
(introduced by J.F. MITCHELL)

Department of Pharmacology, University of Bristol, The Medical School, Bristol BS8 1TD.

On microelectrophoretic administration around mammalian central neurones, D-homocysteate appears to be a more potent and longer-acting excitant than L-homocysteate (Curtis & Watkins, 1963). In potency and in time course of action, the latter amino acid resembles L-glutamate, which is well known to be actively taken up by central nervous tissue. Since the time course of concentration changes around receptor sites may be influenced by such uptake systems, and thus affect the excitation characteristics of an amino acid, a study has been conducted on the relative rates of uptake of D- and L-homocysteate by rat brain slices.

[^{35}S]-L-Homocysteic acid was prepared by oxidation of [^{35}S]-L-homocysteine thiolactone with bromine. Unlabelled D- and L-homocysteic acids were prepared in a similar way from the appropriate isomers of homocystine (Watkins, 1962). Rat cerebral cortex slices were preincubated for 40 min at 37°C in Krebs bicarbonate medium (mM: NaCl, 124; KCl, 5; KH_2PO_4 1.2; $MgSO_4$ 1.3; $CaCl_2$ 2.8; $NaHCO_3$, 26; D-glucose 10, adjusted to pH 7.4). The slices were transferred to fresh medium containing various concentrations of amino acids and further incubated at 37°C for periods of 0 to 20 minutes. The tissue content of [^{35}S]-L-homocysteate was estimated directly by scintillation counting of the hyamine-digested slices, and unlabelled D-, L- or DL-homocysteate were determined by ninhydrin reaction after ion exchange separation from endogenous amino acids. Metabolism of [^{35}S]-L-homocysteate was assessed by high voltage paper electrophoresis and by paper chromatography of an aqueous ethanol extract of the tissue.

On incubation of brain slices with unlabelled D- or L-homocysteate (2.5 mM) for 10 min, only the L form was actively accumulated. Two transport systems for [^{35}S]-L-homocysteate were recognized, these exhibiting low and high affinity characteristics (apparent Km 7 ± 2 mM and 10 ± 3 μM; Vmax 4 ± 1 μmol. g wet wt.$^{-1}$ min^{-1} and 8 ± 3 nmol. g wet wt.$^{-1}$ min^{-1}, respectively). Low affinity uptake of [^{35}S]-L-homocysteate (0.1 mM) was inhibited 20–50% by 1 mM L-glutamate, D-glutamate, L-aspartate and D-aspartate, and 61% by 1 mM *p*-chloromercuriphenylsulphonate. D-Homocysteate (1 and 10 mM) did not

significantly inhibit low affinity uptake. High affinity uptake of [^{35}S]-L-homocysteate (0.1 μM) was not significantly inhibited by 0.1 mM D- or L-glutamate, D- or L-aspartate or D-homocysteate, but was inhibited 70% by 0.1 mM *p*-chloromercuriphenylsulphonate. At 0°C, low affinity uptake as reduced by more than 90% and high affinity uptake was abolished. No metabolism of [^{35}S]-L-homocysteate (0.1 mM) was detected (37°C/10 min).

These results provide a possible explanation for the different characteristics of neuronal excitation produced by D- and L-homocysteate. The functional significance of the energy-dependent uptake systems for L-homocysteate, the high affinity component of which appears to possess a high degree of stereospecificity, remains to be investigated.

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References

CURTIS, D.R. & WATKINS, J.C. (1963). Acidic amino acids with strong excitatory actions on mammalian neurones. *J. Physiol. (Lond.)*, **166**, 1–14.

WATKINS, J.C. (1962). The synthesis of some acidic amino acids possessing neuropharmacological activity. *J. Med. (Pharm.) Chem.*, **5**, 1187–1199.

The response of the rat anococcygeus muscle to stimulation of the individual extrinsic nerves and its modification by drugs

H.C. McKIRDY & T.C. MUIR

Institute of Physiology and Department of Pharmacology, Glasgow University, Glasgow G12 8QQ, Scotland.

Two extrinsic nerves supply the rat anococcygeus (Gillespie & Lullman-Rauch, 1974). These nerves subdivide and supply a nerve plexus comprising ganglia and interconnecting tracts. In this study the response of the muscle to stimulation of each of the two extrinsic nerves has been compared with that to stimulation of the combined extrinsic nerve supply and that to field stimulation (Gillespie, 1972).

Each nerve (one a branch of the external spermatic division of the genito-femoral nerve and the other a branch of the posterior scrotal division of the perineal nerve) was stimulated individually (1–100 Hz for 5 s, 0.3 ms, supramaximal) with suction electrodes positioned 0.5–1 cm from the muscle which was perfused with a modified saline solution (Creed, Gillespie & Muir, 1975).

A contraction (latency 0.5–2.5 s) was produced on stimulation of either nerve (optimal frequency about 50 Hz); the contraction following stimulation of the external spermatic branch was usually the larger. No contraction was produced on stimulation of either nerve with a single stimulus or indeed below 2 Hz. The excitatory response was abolished by tetrodotoxin (TTx, 1 μg/ml) or by phentolamine mesylate (Ph) (10 μg/ml) confirming earlier suggestions (Gillespie, 1972) that it was mediated via an adrenergic nerve pathway. The excitatory response was reduced by the ganglion blocking agents, tubocurarine chloride (Tc, 0.1 mg/ml) or hexamethonium bromide (C_6, 0.5 mg/ml).

When the tone of the muscle was raised (e.g. following carbachol chloride (2 μg/ml) with or without subsequent Ph (10 μg/ml), or following guanethidine sulphate (9 μg/ml) or in a few instances in the absence of any drug) stimulation produced an inhibitory response in about half the experiments. The most obvious explanation for the absence of inhibitory responses in some muscles is that the fibres were missed or damaged in the dissection. When present, the inhibitory responses resulted from stimulation of either or both extrinsic nerves. The inhibitory response which had a variable latency (1.5–5 s) was abolished by TTx (1 μg/ml). The inhibitory response (optimal at about 10 Hz) was not reduced and indeed in some cases was increased in amplitude and/or prolonged by either C_6, or Tc (up to 1 mg/ml) or atropine sulphate (up to 1 μg/ml).

The present evidence, in contrast to previous views (Gillespie, 1972) suggests that synaptic interruption occurs in the excitatory pathway—probably in the ganglionic plexus lying close to the muscle. Two puzzling features of the inhibitory response require explanation—its long latency and the effects of acetylcholine antagonists. The organization of the inhibitory pathway remains obscure.

References

CREED, K.E., GILLESPIE, J.S. & MUIR, T.C. (1975). The electrical basis of excitation and inhibition in the rat anococcygeus muscle. *J. Physiol. (Lond.)*, **245**, 33–47.

GILLESPIE, J.S. (1972). The rat anococcygeus muscle and its response to nerve stimulation and to some drugs. *Br. J. Pharmac.*, **45**, 404–416.

GILLESPIE, J.S. & LULLMAN-RAUCH, R. (1974). On the ultrastructure of the rat anococcygeus muscle. *Cell. Tiss. Res.*, **149**, 91–104.