can be reproduced in adult fowls by its infusion into the IIIrd ventricle or hypothalamus; this and the fall of body temperature were due to an action of clonidine on brain α-adrenoreceptors. Although the effects resembled those of noradrenaline given intraventricularly or infused into the hypothalamus (Grunden & Marley, 1970; Marley & Stephenson, 1970; Marley & Nisticò, 1972), there were differences inasmuch as intraventricular clonidine much more obviously activated heat-loss mechanisms (panting, wing abduction and long-lasting peripheral vasodilatation).

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References

FELDBERG, W. & SAXENA, P.N. (1971). Effects of adrenoreceptor blocking agents on body temperature. *Br. J. Pharmac.*, 43, 543-554.

GRUNDEN, L.R. & MARLEY, E. (1970). Effects of sympathomimetic amines injected into the third cerebral ventricle in adult chickens. *Neuropharmacology*, 9, 119-128.

HOLMAN, R.B., SHILLITO, E.E. & VOGT, M. (1971). Sleep produced by clonidine (2-(2,6-dichlorophenyl-amino)2-imidazoline hydrochloride). *Br. J. Pharmac.*, 43, 685-695.

MARLEY, E. & NISTICÒ, G. (1972). Effects of catecholamines and adenosine derivatives given into the brain of fowls. *Br. J. Pharmac.*, 46, 619-636.

MARLEY, E. & STEPHENSON, J.D. (1970). Effects of catecholamines infused into the brain of young chickens. *Br. J. Pharmac.*, 40, 639-658.

MASKREY, M., VOGT, M. & BLIGH, J. (1970). Control effects of clonidine (2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride, St 155) upon thermoregulation in the sheep and goat. *Eur. J. Pharmacol.*, 12, 297-302.

ZAIMIS, E. (1970). The pharmacology of Catapres (St 155). In: *Catapres in Hypertension*, ed. Conolly, M.E., pp. 9-22, London: Butterworth.

Synaptosomal exchange of γ-amino-butyric acid (GABA) can simulate high affinity uptake

G. LEVI* & M. RAITERI

Laboratorio di Biologia Cellulare, CNR, Via Romagnosi 18/A, Roma, and Istituto di Farmacologia, Università Cattolica, Roma, Italy

It is generally believed that inactivation of neurotransmitter amino acids occurs through high affinity uptake systems present in presynaptic nerve terminals (Iversen & Johnston, 1971; Snyder, Young, Bennett & Mulder, 1973). These uptake systems were demonstrated by measuring the radioactivity taken up by various nervous tissue preparations (including synaptosomes) from incubation media containing micromolar concentrations of radio-labelled amino acids. Since brain contains millimolar concentrations of the amino acids studied, exchange could account for the high tissue/medium ratios of radioactivity obtained. This possibility was underestimated in previous studies (Iversen & Neal, 1968; Levi, Bertollini, Chen & Raiteri, 1974).

Purified synaptosomes prepared from adult Wistar rat cerebrum (Gray & Whittaker, 1962) were prelabelled by incubating them for 10 min in a Krebs-Ringer medium containing 0.5 μM [^{3}H]-GABA and 0.1 mM aminooxyacetic acid. Spontaneous release and exchange of [^{3}H]-GABA were studied by a superfusion technique which prevents re-uptake of released substrates (Raiteri, Angelini & Levi, 1974). The following results were obtained: (1) Superfusion with unlabelled GABA (1-1000 μM) enhanced the release of [^{3}H]-GABA from prelabelled synaptosomes in a concentration dependent, saturable way. A concentration of GABA as low as 1 μM doubled the efflux of [^{3}H]-GABA. (2) The calculated exchange rates obtained with 1-20 μM unlabelled GABA were similar to the initial rates of [^{3}H]-GABA high affinity uptake reported previously (Levi & Raiteri, 1973). In particular, the exchange rate at a saturating GABA concentration (1 mM) almost coincided with the apparent *Vmax* of the high affinity uptake system. (3) Exchange showed a high substrate specificity, similar to that described for uptake. (4) In the absence of sodium, a condition which inhibits most of the high affinity uptake of GABA, homoexchange became undetectable even at high (1 mM) GABA concentrations. (5) When synaptosomes were incubated for 10 min in a medium containing 1, 5 or 10 μM [^{3}H]-GABA, to measure uptake in the concentration range of the high affinity system, the radioactivity decreased in the medium (−49%, −46% and −36% respectively); however, GABA concentrations did not decrease (final concentrations found: 2.1 ± 0.4, 5.5 ± 0.4, and 9.6 ± 0.9 μM respectively; averages of 4-12 determinations) as one would expect if net uptake were the main phenomenon measured. (6) With glycine, exchange at low concentrations (10 and 25 μM) was detectable only in synaptosomes prepared from

areas (spinal cord, brain stem) in which a high affinity uptake for glycine was described (Johnston & Iversen, 1971; Snyder *et al.*, 1973) and where glycine acts as a neurotransmitter (Aprison, Davidoff & Werman, 1970; Curtis & Johnston, 1970).

In conclusion, a process of exchange accounts, at least in large part, for what has been interpreted as high affinity uptake of GABA, and perhaps of other putative neurotransmitter amino acids.

References

APRISON, M.H., DAVIDOFF, R.A. & WERMAN, R. (1970). Glycine: its metabolic and possible transmitter roles in nervous tissue. In: *Handbook of Neurochemistry*, ed. Lajtha, A. Vol. 3, pp. 381-397. New York and London: Plenum Press.

CURTIS, D.R. & JOHNSTON, G.A.R. (1970). Amino acid transmitters. In: *Handbook of Neurochemistry* ed. Lajtha, A., Vol. 4, pp. 115-134, New York and London: Plenum Press.

GRAY, E.G. & WHITTAKER, V.P. (1962). The isolation of nerve endings from brain: an electron microscope study of cell fragments derived by homogenization and centrifugation. *J. Anat., Lond.*, 96, 79-87.

IVERSEN, L.L. & JOHNSTON, G.A.R. (1971). GABA uptake in rat CNS: comparison of uptake in slices and homogenates and the effects of some inhibitors. *J. Neurochem.*, 18, 1939-1950.

IVERSEN, L.L. & NEAL, M.J. (1968). The uptake of [^{3}H]-GABA by slices of rat cerebral cortex. *J. Neurochem.*, 15, 1141-1149.

JOHNSTON, G.A.R. & IVERSEN, L.L. (1971). Glycine uptake in rat central nervous system slices and homogenates: evidence for different uptake systems in spinal cord and cerebral cortex. *J. Neurochem.*, 18, 1951-1961.

LEVI, G., BERTOLLINI, A., CHEN, J. & RAITERI, M. (1974). Regional differences in the synaptosomal uptake of ^{3}H-γ-aminobutyric acid and ^{14}C-glutamate and possible role of exchange processes. *J. Pharmacol. exp. Ther.*, 188, 429-438.

LEVI, G. & RAITERI, M. (1973). Detectability of high and low affinity uptake systems for GABA and glutamate in rat brain slices and synaptosomes. *Life Sci. Part 1*, 12, 81-88.

RAITERI, M., ANGELINI, F. & LEVI, G. (1974). A simple apparatus for studying the release of neurotransmitters from synaptosomes. *Eur. J. Pharmacol.*, 25, 411-414.

SNYDER, S.H., YOUNG, A.B., BENNETT, J.P. & MULDER, A.H. (1973). Synaptic biochemistry of amino acids. *Fed. Proc.*, 32, 2039-2047.

On the release of accumulated [^{3}H]-γ-aminobutyric acid (GABA) from isolated rat superior cervical ganglia.

N.G. BOWERY* & D.A. BROWN

Department of Pharmacology, The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX

Exogenous GABA is accumulated by glial cells in rat superior cervical ganglia (Bowery & Brown, 1972; Young, Brown, Kelly & Schon, 1973). Some preliminary observations (Bowery & Brown, 1972) suggested that labelled GABA taken up by this tissue could be released by 'depolarizing' stimuli. In the present experiments we have examined this phenomenon more closely.

Superior cervical ganglia (with pre- and post-ganglionic trunks) were excised from male Wistar rats (anaesthetized with urethane 1.5 gm/kg), desheathed, and incubated for 2-3 h in [^{3}H]-GABA (0.1-20 μM) in the presence of amino-oxyacetic acid (AOAA, 10 μM). The ganglia were then superfused at 0.5 ml/min with Krebs solution at ~22° C in such manner that the effluent radioactivity from the entire preparation or from individual regions (pre-ganglionic nerve trunk, post-ganglionic trunk or ganglion soma) could be collected at 2 min intervals and measured.

After 15-20 min superfusion of the entire preparation the release of tritium assumed a single exponential over several hours with a rate coefficient of 0.0014 ± 0.0002/min (mean ± s.e. of 27 ganglia). This value was independent of the ganglionic [^{3}H]-GABA concentration between 2 and 364 μM. In the presence of AOAA, released tritium (resting and stimulated) as well as accumulated tritium (Bowery & Brown, 1972; Walsh, Bowery, Brown & Clark, 1974) corresponded to >95% [^{3}H]-GABA. This rate coefficient for release was increased (a) by raising external [K^+] (the increment in rate coefficient increasing with $[K]_0$ over the range 30-140 mM); and (b) by applying electrical stimuli to the ganglion soma or pre-ganglionic nerve trunk (threshold 0.5 Hz, 0.1 ms duration).

These 'depolarizing' stimuli are comparable to those previously found to release GABA from presumed neural loci in brain tissue (Srinivasan, Neal & Mitchell, 1969; Katz, Chase & Kopin, 1969). Accelerated release following orthodromic stimulation is especially interesting because such stimuli also produce depolarization of ganglionic