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Muscimol and the Uptake of γ-Aminobutyric Acid by Rat Brain Slices

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Abstract. Muscimol is a weak, non-competitive inhibitor (K_i 1.2×10^{-3} M) of the uptake of γ-aminobutyric acid by slices of rat cerebral cortex.

Key-Words: Muscimol — Amanita Muscaria — γ-Aminobutyric Acid.

Muscimol (Fig. 1), a psychotomimetic isolated from the mushroom *Amanita muscaria* (Eugster, 1967; Waser, 1967), is a structural analogue of γ-aminobutyric acid (GABA), an inhibitory synaptic transmitter of major significance in the mammalian central nervous system (Curtis and Johnston, 1970). Both muscimol and GABA are powerful inhibitors of the firing of central neurones when administered by microelectrophoresis (Johnston *et al.*, 1968) and the inhibition induced by these compounds can be anatagonised by the convulsant alkaloid bicuculline (Curtis *et. al*, 1970). Unlike GABA, however, muscimol exerts pronounced central effects when administered systemically to healthy adult mammals (Waser, 1967; Theobald *et al.*, 1968; Scotti de Carolis *et al.*, 1969). A high affinity uptake system for GABA has been described in CNS tissue slices (Iversen and Neal, 1968), which concentrates GABA predominantly into nerve terminals (Neal and Iversen, 1969; Bloom and Iversen, 1971), and which may be important for the removal of GABA from the extracellular synaptic environment (Curtis *et al.*, 1970). The present investigation is concerned with the influence of muscimol and some related compounds on this GABA uptake system.

The uptake of ^{3}H-GABA (specific activity 2.0 Ci/mmol, New England Nuclear Chemicals GmbH, Germany) by slices of rat cerebral cortex during 10 min at 25° C was studied using the method of Iversen and Neal (1968). Muscimol and derivatives were kindly provided by Dr. P. Hackett of the Woodstock Agricultural Research Centre, Sittingbourne, Kent.

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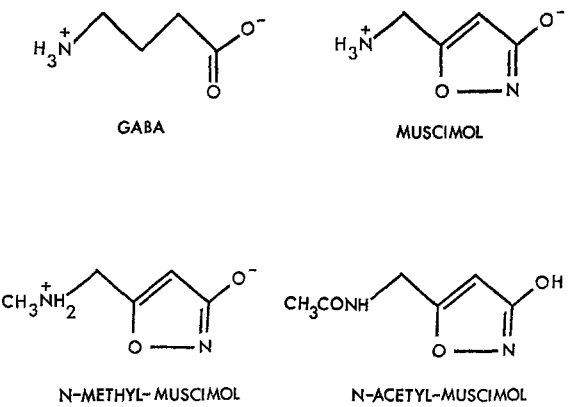

Fig. 1. Structural formulae for GABA, muscimol and derivatives

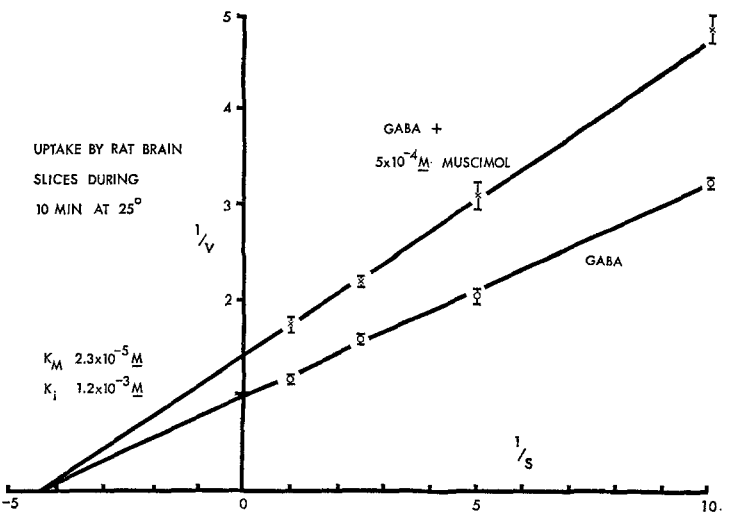

Fig. 2. Kinetic analysis of muscimol-induced inhibition of GABA uptake in slices of rat cortex. Uptake of ^{3}H-GABA (V, μmoles/g/min) was determined after incubation of cortical slices for 10 min in media containing various concentrations of GABA (S, 10^{-4} M) in the presence and absence of 5×10^{-4} M muscimol. Each point is the mean ± S.E. of 4 determinations

Muscimol proved to be a relatively weak, but specific, inhibitor of GABA uptake. Kinetic analysis of this inhibition using a Lineweaver-Burk plot (Fig. 2) indicated that muscimol-induced inhibition was non-competitive in nature with a K_i of 1.2×10^{-3} M, compared to a K_M for GABA of 2.3×10^{-5} M. Muscimol (5×10^{-4} M) did not significantly

influence the uptake into brain slices of any of the following probable central synaptic transmitters: L-aspartic acid, L-glutamic acid, glycine, 5-hydroxytryptamine and (+)-noradrenaline. The uptake of glycine by slices of rat spinal cord (Neal and Pickles, 1969) was also not influenced by muscimol. Derivatives of muscimol in which the primary amino function is acetylated or methylated (Fig. 1) were inactive against GABA uptake at concentrations of 5×10^{-4} M. The inhibition of GABA uptake by muscimol was reversible, as shown by washout studies, and pre-incubation of the tissue with muscimol for up to 30 min before the addition of GABA did not increase the extent of inhibition.

The interaction of muscimol with the GABA uptake system is weak compared to that of some other structural analogues of GABA, e.g. α-amino-, α-fluoro- and β-hydroxy-GABA show K_i's of less than 10^{-4} M as inhibitors of GABA uptake (Iversen and Johnston, 1971). Since a substance transported by the GABA uptake system would be expected to significantly inhibit GABA uptake when present in sufficient molar excess, it is concluded that muscimol can be transported only inefficiently by the GABA system and not at all by the uptake systems for the other probable transmitters studied. Inefficient removal of muscimol from the environment of GABA receptors may be an important factor contributing to the central effects of this psychotomimetic.

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References

Bloom, F. E., Iversen, L. L.: Localizing ^{3}H-GABA in nerve terminals of rat cerebral cortex by electron microscopic autoradiography. Nature (Lond.) **229**, 628—630 (1971).

Curtis, D. R., Duggan, A. W., Felix, D., Johnston, G. A. R.: GABA, bicuculline and central inhibition. Nature (Lond.) **226**, 1222—1224 (1970).

— — Johnston, G. A. R.: The inactivation of extracellularly administered amino acids in the feline spinal cord. Exp. Brain. Res. **10**, 447—462 (1970).

— Johnston, G. A. R.: Amino acid transmitters. In: Handbook of Neurochemistry. vol. 4, pp. 115—134. A. Lajtha, ed. New York: Plenum Press 1970.

Eugster, C. H.: Isolation, structure and syntheses of central-active compounds from Amanita muscaria (L. ex Fr.) Hooker. In: Ethnopharmacologic search for psychoactive drugs, pp. 416—418. D. H. Efron, B. Holmstedt, N. S. Kline, eds. Washington: U.S. Public Health Service publication no. 1645 (1967).

Iversen, L. L., Johnston, G. A. R.: GABA uptake in rat CNS: comparison of uptake in slices and homogenates and the effects of some inhibitors. J. Neurochem. (accepted for publication) (1971).

— Neal, M. J.: The uptake of (^{3}H)GABA by slices of rat cerebral cortex. J. Neurochem. **15**, 1141—1149 (1968).

Johnston, G. A. R., Curtis, D. R., de Groat, W. C., Duggan, A. W.: Central actions of ibotenic acid and muscimol. Biochem. Pharmacol. **17**, 2488—2489 (1968).

Neal, M. J., Iversen, L. L.: Subcellular distribution of endogenous and (^{3}H)-γ-aminobutyric acid in rat cerebral cortex. J. Neurochem. **16**, 1245—1252 (1969).

— Pickles, H. G.: Uptake of ^{14}C-glycine by spinal cord. Nature (Lond.) **222**, 679—680 (1969).

Scotti de Carolis, A., Lipparini, F., Longo, V. G.: Neuropharmacological investigations on muscimol, a psychotropic drug extracted from Amanita muscaria. Psychopharmacologia (Berl.) **15**, 186—195 (1969).

Theobald, W., Buch, O., Kunz, H. A., Krupp, P., Stenger, E. G., Heimann, H.: Pharmakologische und experimental-psychologische Untersuchungen mit 2 Inhaltsstoffen des Fliegenpilzes (Amanita muscaria). Arzneimittel-Forsch. **18**, 311—315 (1968).

Waser, P. G.: The pharmacology of Amanita muscaria. In: Ethnopharmacologic search for psychoactive drugs, pp. 419—439. D. H. Efron, B. Holmstedt, N. S. Kline, eds. Washington: U.S. Public Health Service publication no. 1645 (1967).

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