reduced below the now-elevated threshold for spike generation. In support of this hypothesis, prolonged depolarization (produced by either injected current or carbachol) elevated the voltage threshold and reduced the spike overshoot and rise rate as expected for increased resting Na^+-inactivation (Hodgkin & Huxley, 1952). Another consequence of pronounced Na-inactivation is the failure of these cells to give repetitive spikes during prolonged depolarization.

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References

ADAMS, P.R. & BROWN, D.A. (1973). Action of γ-aminobutyric acid (GABA) on rat sympathetic ganglion cells. *Br. J. Pharmac.*, 47, 639-640P.

ADAMS, P.R. & BROWN, D.A. (1975). Actions of γ-aminobutyric acid on sympathetic ganglion cells. *J. Physiol.* (in press).

DE GROAT, W.C. (1970). Actions of γ-aminobutyric acid and related amino acids on mammalian autonomic ganglia. *J. Pharm. exp. Ther.*, 172, 384-396,

HODGKIN, A.L. & HUXLEY, A.F. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.*, 117, 500-544.

L-2,4-diaminobutyric acid (L-DABA) as a selective marker for inhibitory nerve terminals in rat brain

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^{3}H-GABA is accumulated in glial cells of rat sensory ganglia by a high affinity uptake process, which like that in nerve terminal is temperature sensitive and requires the presence of sodium ions in the incubation media, (Schon & Kelly, 1974a and b). Furthermore, ^{3}H-GABA uptake into glial cells was shown to be potently inhibited by β-alanine, a poor inhibitor of ^{3}H-GABA uptake in nerve terminals. In contrast, another GABA analogue, L-DABA was a potent inhibitor of ^{3}H-GABA uptake into nerve terminals but was less effective on the uptake of ^{3}H-GABA into glia. Schon & Kelly (unpublished observations) later confirmed that β-alanine was a substrate for the GABA uptake process in glia of the rat sensory ganglia and cerebral cortex and suggested that this amino acid might prove useful as a specific maker for such glial sites. We have examined the alternative possibility that L-DABA is a specific substrate for the GABA uptake process in nerve terminals (Simon & Martin, 1973) but not for that in glial cells, and that L-DABA might thus prove useful in the identification of nerve terminals able to take up GABA.

This hypothesis was confirmed directly by using ^{3}H-DL-DABA. The accumulation of ^{3}H-DL-DABA into small prisms of rat cerebral cortex continued linearly for one h and appeared to be mediated by a saturable process with an apparent K_m of 31 μM and a V_{max} of 33 nmol g^{-1} min^{-1} at 25°C. Like the uptake of GABA, the uptake of DABA was sodium dependent, however an increase in the incubation temperature from 25°C to 37°C greatly enhanced the uptake of DABA but not that of GABA. DABA accumulation also exhibits the same chemical specificity as that for GABA and was potently inhibited by GABA (IC50 = 17 μM) and the GABA analogues, DL-3 hydroxy GABA (IC50 = 100 μM) and 3-fluoro-GABA and the mercurial, *p*-chloromercuriphenylsulphonic acid (IC50 = 19 μM) and unaffected by β-alanine (Test concentration = 1 mM).

Electron microscopic autoradiography showed that the ^{3}H-DABA accumulated by small prisms of cerebral cortex was localized predominantly over nerve terminals. Furthermore, when ^{3}H-DABA was injected from a fine glass microelectrode into a single lobule of the rat cerebellar vermis and the animal killed by perfusion with 5% glutaraldehyde in Krebs' solution, electron microscopic autoradiography showed labelling over small nerve terminals and neurone cell bodies in all layers of the cerebellum. More detailed analysis allowed many of the labelled constituents to be identified either as stellate cells, or axon terminals of Golgi or basket cells.

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References

SCHON, F. & KELLY, J.S. (1974a). The characterization of ^{3}H-GABA uptake into satellite glial cells of rat sensory ganglia. *Brain Research*, 66, 289-300.

SCHON, F. & KELLY, J.S. (1974b). Autoradiographic localization of ^{3}H-GABA and (^{3}H)-glutamate over satellite glial cells. *Brain Research*, 66, 275-288.

SIMON, J.R. & MARTIN, D.L. (1973). The effects of L-2,4-diaminobutyric acid on the uptake of gamma aminobutyric acid by a synaptosomal fraction from rat brain. *Arch. Biochem. Biophys.*, 157, 348-355.