The superfusion of the lobster fibre with glutamate ($0.5\text{-}1.5 \times 10^{-4}$ M) produced membrane depolarizations which reached a maximum within 2 min (Hironaka, 1974). Depolarizations induced by Kai (10^{-4} to 10^{-3} M) were smaller than those caused by glutamate, the action of which was potentiated by Kai in concentrations producing little or no depolarization alone (Figure 1).

In the frog cord, glutamate (5×10^{-5} to 10^{-3} M) and Kai (in lower concentrations of 10^{-5} to 10^{-4} M) evoked spike activity in the ventral roots with initial increase and subsequent depression of ventral root potentials. In the first 10 min glutamate (10^{-4} M) induced a 51% increase in the spinal ACh output while with Kai (10^{-4} M) a 112% rise was found. In still lower concentrations, neither Kai (5×10^{-6} M) nor glutamate (5×10^{-5} M) separately affected ACh output but when added simultaneously raised the ACh output by 30%.

These findings suggest that Kai has more potent effects than glutamate on the frog spinal cord but not on the lobster muscle fibre.

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Actions of γ-aminobutyric acid (GABA) on ganglionic transmission and ganglion cell excitability

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GABA increases Cl^- conductance in rat sympathetic ganglion cells and produces a membrane depolarization, probably because $E_{Cl} < E_m$ (Adams & Brown, 1973). We wish here to consider the consequences for ganglion cell excitability and transmission.

Isolated rat superior cervical ganglia were bathed in flowing oxygenated Krebs' solution at ambient temperature, and neurones impaled with microelectrodes filled with K citrate or acetate. Cells were stimulated directly by passing depolarizing current pulses through the microelectrode or orthodromically through a suction electrode into which the preganglionic trunk was drawn.

On the direct spike GABA (100 μM) (i) reduced the positive overshoot and rate of rise and (ii) increased the threshold depolarizing current. These effects could be explained quantitatively in terms of (a) the increased membrane conductance and (b) increased steady-state Na^+-inactivation during GABA-depolarization (Hodgkin & Huxley, 1952).

In neurones showing minimal impalement shunting (such that the recorded membrane potential exceeded −55 mV (Adams & Brown, 1975)) GABA did not usually block the transmission of single, supramaximal orthodromic stimuli: instead, the amplitude of the orthodromic spike was reduced to an extent comparable with that of the direct spike. This accords with previous observations with extracellular electrodes (De Groat, 1970; Bowery & Brown, unpublished observations). Although the amplitude of the synaptic potential is severely depressed by GABA, transmission does not fail because the synaptic potential is superimposed on a depolarization driving toward E_{Cl} such that the membrane potential attained by the synaptic potential still exceeds the threshold for spike generation.

In contrast, when the impalement leak short-circuited the membrane resistance sufficiently to reduce the resting potential below −55 mV, orthodromic transmission was invariably blocked by GABA. This might be an experimental artefact, reflecting increased Na^+-inactivation during the sustained impalement-depolarization with a consequent rise in the voltage threshold for spike generation. Thus, although attaining a lower absolute membrane potential than in undamaged cells, on adding GABA the synaptic potential is

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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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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