displacement caused by passing a hyperpolarizing current pulse through the recording electrode by means of a bridge circuit. Cells which responded to met-enkephalin also responded to normorphine (300 nM–1 μM); cells which were unaffected by met-enkephalin were also unaffected by normorphine. Leu-enkephalin had qualitatively similar effects but appeared to be less potent. The hyperpolarization produced by met-enkephalin was reversed by changing to a solution which contained both met-enkephalin and naloxone (20–50 nM); exposure to naloxone alone did not affect the membrane potential but it prevented the action of enkephalin.

Type 2 cells seldom showed any effects of met-enkephalin. A few cells showed a small hyperpolarization (2–5 mV) which passed off during the continued presence of the met-enkephalin.

The functional significance of these effects in the myenteric plexus is not clear. Although the myenteric plexus contains enkephalins (Elde, Hökfelt, Johansson & Terenius, 1976; Smith, Hughes & Kosterlitz, 1976) inhibitory synaptic potentials have not been observed in this tissue. On the other hand, the hyperpolarization provides a basis for the inhibition of spike firing by enkephalins.

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Intracellular recording of the effects of 5-hydroxytryptamine on rabbit superior cervical ganglion cells

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The complex actions of 5-hydroxytryptamine (5-HT) at sympathetic ganglia, which include facilitation and depression of transmission and ganglion cell depolarization (see Wallis & Woodward, 1974), are difficult to elucidate by examining the response of a population of cells. In the work reported here, intracellular recordings were made from rabbit superior cervical ganglia. Rabbits were killed by air embolism and the excised ganglia superfused with Krebs solution at 37°C. Microelectrodes were filled with 2M KCl.

Superfusion of the whole ganglion with 5-HT (10^{-6}–10^{-4}M) produced only small or negligible changes in resting membrane potential and membrane resistance. In 24 tests on 20 cells, 5-HT had no apparent effect on nine occasions, caused a small depolarization (< 5 mV) on eleven occasions and a small hyperpolarization on four occasions. The first application of 5-HT more often produced effects than subsequent ones, suggesting the variability in responsiveness might be related to the rapid tachyphylaxis to 5-HT previously reported for these cells (Wallis & Woodward, 1975).

On the other hand, iontophoresis of 5-HT on to the ganglion cell membrane induced substantial depolarizations with a fall in membrane resistance in 29 of 41 cells tested. The iontophoresis electrode was filled with 20 mM 5-HT creatinine sulphate and positioned near an impaled cell, using a separate micromanipulator. Some cells responded to ejection currents of 20 nA for 100 ms, others only after passing greater currents for several seconds. Only outward current evoked depolarizations. Twelve cells were apparently unresponsive to 5-HT. Lack of response was not easy to establish unequivocally because of difficulty in visualizing the position of the iontophoresis electrode with respect to the impaled cell.

Iontophoresis of 5-HT induced depolarizations which were graded in amplitude (up to 19 mV) as the duration or the intensity of the ejection current was altered. The time to peak of the depolarization was typically 1–3 seconds. Membrane resistance fell by 15–55% at the peak of the depolarization. An after-hyperpolarization sometimes followed the depolarization by which time membrane resistance had returned to control values. The depolarization was sometimes clearly divided into an initial and a late depolarization, evident as a hump on the falling phase of the initial response. The 5-HT depolarization did not decline in

amplitude when elicited at frequencies of 0.033 Hz; at 0.1 Hz the amplitude of second and subsequent responses declined by 25–30%. The amplitude of the 5-HT depolarization was augmented by hyperpolarization and decreased by depolarization of the soma membrane of the ganglion cell.

Iontophoretic currents were not exciting nerve terminals and releasing acetylcholine because (a) iontophoretic responses were unaffected by hexamethonium (5×10^{-4} M), whereas synaptic potentials were depressed by 70%; (b) responses were reduced in amplitude by cyproheptadine (5×10^{-5} M) by about 50%; (c) responses were reduced in amplitude by superfusing the ganglion with 10^{-6} M 5-HT and nearly abolished on superfusing with 5×10^{-5} M 5-HT, although these concentrations of 5-HT did not affect the synaptic potentials.

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The uptake of ^{45}calcium into slices of rat cerebral cortex: effect of depolarizing stimuli

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Previous studies have shown that neurotransmitter uptake is markedly dependent on slice size (Levi & Raiteri, 1973) and it has been postulated that varying slice size can be used to examine uptake into neuronal and glial cells (Riddall, Leach & Davison, 1976; Leach, Riddall & Winkley, 1976). The intracellular concentration of calcium may be increased by various depolarizing stimuli and the effects of these stimuli on ^{45}calcium uptake into slices of rat cerebral cortex has been examined.

Two sizes of slice were employed ($0.1 \times 0.1 \times 2$ mm and $0.4 \times 0.4 \times 2$ mm) and they were prepared in the manner described by Iversen & Neal (1968) except that pH 7.4 Tyrode medium was used. Slices were pre-incubated at 37°C for 15 min and portions (equivalent to 20 mg wet wt.) of this suspension were transferred to Tyrode (containing the depolarizing stimuli) or to potassium stimulation media both of which contained $^{45}Ca^{2+}$ (0.1 μCi ml, 1.78 mM). Flasks were incubated for varying times and uptake was terminated by the addition of 1 ml of quench medium (24 mM EGTA-tris, 72 μM ruthenium red in Tyrode). The suspension was immediately filtered under vacuum, washed, and the resulting tissue sample was digested and counted by the method of Dent & Johnson (1974).

The basal flux of $^{45}Ca^{2+}$, i.e. 'exchange', was greater in 0.4 mm slices than in 0.1 mm slices giving asymptotic 'uptake' values of 5.64 ± 0.26 and 1.94 ± 0.11 μmol Ca^{2+}/g wet wt. ($P < 0.01$) respectively. In the presence of ouabain (0.1 mM) the corresponding asymptotic values for $^{45}Ca^{2+}$ uptake into 0.4 mm and 0.1 mm slices were increased to 12.27 ± 0.38 and 3.44 ± 0.23 μmol Ca^{2+}/g wet wt. ($P < 0.01$). Pre-treatment with the calcium ionophore, A23187 (200 μg/ml) increased $^{45}Ca^{2+}$ uptake into both slice sizes at 10 and 60 min of incubation. Glutamate (5 mM) had no effect on $^{45}Ca^{2+}$ uptake into either slice size.

Potassium exhibited different effects on the uptake of $^{45}Ca^{2+}$ into the two slice sizes. K^+ (49 mM) stimulation of $^{45}Ca^{2+}$ uptake into 0.1 mm slices was characterized by a rapid initial phase which lasted for approximately one minute. This rapid initial phase was followed by a slower phase which was similar in magnitude to the uptake found in the absence of elevated K^+. In contrast K^+ (49 mM) had no significant effect on $^{45}Ca^{2+}$ uptake into 0.4 mm slices.

Uptake mechanisms for putative neurotransmitters into small slices ($0.1 \times 0.1 \times 2$ mm) of rat cerebral cortex are well documented. Conversely, there are little, if any, data relating to the release mechanisms operating in these slices. The results presented above indicate a prominent role for glial cells in the regulation of Ca^{2+} flux. This role is demonstrated by the effects of slice size and depolarizing stimuli on $^{45}Ca^{2+}$ uptake into rat cerebral cortex.