Release of acetylcholine and ^{14}C-glycine from the cat spinal cord *in vivo*

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There is considerable evidence to suggest that acetylcholine (ACh) mediates transmission between motoneurone collaterals and Renshaw cells in the spinal cord and that glycine may be an inhibitory transmitter at motoneurones. Release of ACh into the perfused vasculature of the cat cord has been demonstrated (Kuno & Rudomin, 1966), but an equivalent observation has not been made for glycine.

In these experiments the central canal of the spinal cord was perfused in cats (spinalized at C1 after halothane anaesthesia) using a modification of the method described by Morton, Stagg & Webster (1969). A hypodermic needle was lowered into the canal at L4 by means of a micromanipulator, and artificial cerebrospinal fluid (A.C.S.F.) perfused (1 ml/15 min) through the canal and collected via a fine polythene cannula inserted into the canal at S1. Samples were normally collected for periods of either 15 min when studying ACh release or 5–10 min for glycine studies. Both femoral and both sciatic nerves were prepared for simultaneous stimulation, and in some experiments the input to the cord was limited to ventral roots by dorsal root section. The stimulus parameters used were 10 v, 0·5 ms duration pulses at 2–10 Hz applied for 3×2 min periods separated by 1 min rest periods. The animals were curarized to prevent movement during stimulation.

During perfusion with A.C.S.F. (containing eserine $2{\cdot}1\times10^{-5}$ M) stimulation increased the ACh content of the perfusate as measured on the dorsal muscle of the leech. When atropine ($1{\cdot}4\times10^{-7}$ M) was present in the A.C.S.F. both resting ($71{\cdot}9\pm8{\cdot}5$ S.E. of mean pmol/15 min, $n=16$) and stimulated ($168{\cdot}3\pm29{\cdot}1$, $n=9$) levels were higher ($P=<0{\cdot}02$) than when normal CSF was used (resting $44{\cdot}4\pm6{\cdot}3$, $n=15$; stimulated $85{\cdot}4\pm13{\cdot}7$, $n=11$). Section of dorsal roots (two experiments) only slightly reduced the stimulated levels of ACh, implying a substantial release from motoneurone collaterals.

In other experiments, the cord was loaded with ^{14}C-glycine (108 μCi/μ mol) by perfusing with a 1 μM solution in A.C.S.F. for 1 hour. Efflux into glycine-free A.C.S.F. was then monitored by liquid scintillation counting. Stimulation (5 Hz) failed to increase the spontaneous efflux of glycine (normally 300–600 d.p.m./5 min). However, addition to the A.C.S.F. of 10^{-5} M *p*-hydroxymercuribenzoate (*p*-HMB), which reduces the uptake of ^{14}C-glycine into rat cord slices (Neal & Pickles, 1969), increased the spontaneous efflux by 3·3–9·3 times. Stimulation (5 Hz) then resulted in a further maximal increase of 2·1–2·8 times in four out of six experiments. Succeeding periods of stimulation were in all cases less effective, possibly because the *p*-HMB level is a highly critical factor.

These results do not establish the role of glycine as a transmitter in the mammalian spinal cord, but they are consistent with this hypothesis.

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