The release of acetylcholine from rabbit hippocampus

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There is evidence that acetylcholine (ACh) is a synaptic transmitter substance in many areas of the mammalian central nervous system. Although the release of ACh has been demonstrated from the surface of the cerebral and cerebellar cortices, the caudate nucleus, the medulla and other areas, the demonstration of release from deep structures presents many difficulties. Such studies involve either perfusion of the ventricles where there is uncertainty of the origin of the released ACh or push-pull cannulae where serious damage of the tissue may occur. In an attempt to avoid these problems, the release of ACh from the hippocampus has been investigated using a modification of the cortical cup technique (MacIntosh & Oborin, 1953; Mitchell, 1963).

The hippocampus was chosen for the present study as there is evidence of a well defined afferent and probably cholinergic pathway from the septum to the hippocampus (Lewis, Shute & Silver, 1967).

Rabbits were anaesthetized with urethane. The cortex overlying the septum and hippocampus was removed by suction and a small perspex cylinder covering 0·25 cm^2 was placed on the dorsal surface of the hippocampus. Ringer-Locke solution (0·5 ml) containing eserine sulphate (10^{-4} g/ml) and atropine sulphate (10^{-6} g/ml) prewarmed to 37° C, was placed in the cylinder. ^{14}C-urea (10 μCi) was then added to the cup fluid and 45 min allowed for equilibration. After this period successive 15 or 20 min samples were taken and assayed for ACh, using the eserinized dorsal muscle of the leech.

Under these conditions there was a spontaneous resting release of ACh from the hippocampus which in 6 rabbits varied from 0·31–1·25 (ng/15 min)/0·25 cm^2 (mean $=0·89 \pm$ S.E. 0·17). However the release of ACh in successive samples within an experiment did not usually vary significantly. Stimulation of the surface of the septum (1 ms duration pulses, 1–3 mA, 100 Hz) for 5 min, produced an increase in ACh release of 1·9–3·1 times the control values. (Mean of 6 experiments $=2·4 \pm 0·2$). This evoked release of ACh appears selective as it was not accompanied by an increase in the efflux of the marker substance ^{14}C-urea.

In its response to drugs, the release of ACh from the hippocampus is affected in a similar way to that of the cerebral cortex. Thus topical administration of morphine sulphate (10^{-4} g/ml) into the cup fluid markedly reduced the spontaneous and evoked release of ACh from the hippocampus without affecting the efflux of ^{14}C-urea. Further, the systemic administration of leptazol (30 mg/kg I.V.) increased release. Topical application of atropine sulphate (10^{-6} g/ml) also increased release. Here the release of ACh from cortex was monitored simultaneously and the response to atropine was very similar in both brain areas.

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