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In vivo electrically evoked release of [^{3}H]noradrenaline from cat brain

A neurotransmitter role has been postulated for brain noradrenaline and dopamine. Before a substance can be considered to be a transmitter it must be shown to be released from nerve endings upon depolarizing stimulation. Baldessarini & Kopin (1966) demonstrated that electrical stimulation of brain slices caused the efflux of [^{3}H]noradrenaline, presumably from depolarized nerve endings. Attempts have also been made to detect endogenous dopamine release *in vivo* after electrical stimulation of various brain regions (McLennan, 1964), but the minute amounts of dopamine released were at the limits of the spectrophotofluorometric measurement. Philippu, Heyd & Burger (1970) reported that after the intraventricular injection of [^{14}C] noradrenaline, stimulation of the hypothalamus increased the concentration of ^{14}C (noradrenaline and metabolites) in ventricular perfusates. We now report an increased outflow of [^{3}H]noradrenaline into the ventricular system after electrical stimulation of the caudate nucleus.

Cats (2–3 kg) had their spinal cords sectioned and prepared for cerebroventricular perfusion (Carr & Moore, 1970). Five μCi of [^{3}H]noradrenaline (8·76 Ci/mmol, New England Nuclear Corp.) were injected in an effective volume of 10 μl through a cannula in a lateral ventricle at 16·5 A, 3·5 L (left or right) and + 8·0 D (Snider & Niemer, 1961). After 1 h the ventricular system was perfused with artificial cerebrospinal fluid (Pappenheimer, Heisey & others, 1962) at a rate of 0·1 ml/min. After washout for 110 min, the perfusion rate was increased to 0·5 ml/min and the collection of 1 ml samples of perfusate every 2 min was begun. During one or two of the collection periods, constant current square waves of 1 ms duration, 350 μA intensity and various frequencies were applied to the caudate nucleus by an electrode pair (anode at 13·0 A, 4·0 L and +5·0 D, cathode at 18·0 A, 4·0 L and +5·0 D). [^{3}H]Noradrenaline and metabolites in the perfusates were separated by alumina absorption and ion-exchange chromatography and quantified by liquid scintillation spectrometry (Carr & Moore, 1970). Throughout the course of the experiments blood pressure was recorded from the femoral artery and the rectal temperature monitored and maintained at 37·5° with a heating pad. All cannula and electrode placements were verified by gross dissection of the cat brain after formalin fixation.

In four experiments the mean ($\pm$ s.e.) concentrations of [^{3}H]noradrenaline and [^{3}H]normetanephrine in the perfusate samples before stimulation were 4·6 $\pm$ 1·1 and 2·6 $\pm$ 0·7 nCi/ml respectively. Electrical stimulation for 2 min significantly increased ($P < 0.05$) the perfusate concentrations in the periods during and immediately after the stimulation period (7·4 $\pm$ 1·1); stimulation did not alter the perfusate concentration of [^{3}H]normetanephrine. The other metabolites, deaminated catechols and deaminated *O*-methylated products, were present in the perfusates (10 and 15% of total radioactivity respectively), but they did not increase in concentration during or after stimulation.

When the effects of varying the stimulation frequency from 12·5–100 Hz upon the release of [^{3}H]noradrenaline from the caudate nucleus were examined, it was found that the initial period of stimulation at all frequencies tested in four experiments caused a significant increase ($P < 0.05$) in [^{3}H]noradrenaline perfusate concentration with the greatest release occurring at 50 Hz [increased release of [^{3}H]noradrenaline (nCi/ml) at 12·5 Hz = 0·8 $\pm$ 0·4, 25 Hz = 3·7 $\pm$ 1·6, 50 Hz = 6 $\pm$ 2·1, 100 Hz = 4 $\pm$ 0·7].

These facts support the idea of neurotransmitter roles for brain catecholamines by demonstrating depolarization-evoked release of [^{3}H]noradrenaline; the release is frequency-related, suggesting that it is related to neuronal function.

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Inhibitory action of γ-aminobutyric acid on cryoepilepsy in the frog

The inhibitory action of α-aminobutyric acid (GABA) in the central nervous system synapses has led some authors to investigate its influence on experimental convulsions and correlate its brain content in the central nervous system with convulsive disorders observed (Meynert & Kaji, 1962; Wood & Watson, 1969; Saad, 1970).

We report the action of GABA on an epileptiform attack produced by sudden cooling of the spinal cord of the frog (cryoepilepsy) (Ozorio de Almeida, 1943) and the relation to its content in the nervous centres.

GABA (0·5–5·0 g/kg) was injected into the ventral lymphatic sac of the frog and 1–48 h later the spinal cord was isolated and plunged into a temperature-controlled cooled Ringer bath (Ozorio de Almeida, Moussatché & Vianna Dias, 1941). After the induced convulsive attack, the cords were weighed, homogenized in 1 ml of ice-cold Ringer and centrifuged 15 min at 0° and 15 000 *g*. Free GABA was quantitatively estimated in the supernatant fluid by the *Ascaris lumbricoides* muscle bioassay (Ash & Tucker, 1967, as modified by Moussatché & Cordeiro, unpublished).

The relation between dose, temperature and the inhibition of convulsions is seen in Table 1. Doses of GABA greater than 3·0 g/kg, injected 1–5 h previously, inhibited the convulsions completely when the spinal cord was cooled to 6°; all the controls convulsed. The per cent inhibition of the convulsions is related to the bath temperature (Moussatché & Cuadra, 1967). After 24 or 48 h cords from GABA-treated frogs showed respectively 50%, inhibition or no inhibition when plunged in a bath at 5–6°.