Assay of acetylcholine using toad rectus abdominis muscle in the presence of hemicholinium-like substances

The presence of hemicholinium-3 (HC-3) has been reported to depress the guinea-pig ileum response to acetylcholine, and to a lesser extent, that of the frog rectus abdominis muscle (Prasad & MacLeod, 1966; Bertolini, Greggia & Ferrari, 1967). This action can sometimes prevent the satisfactory assay of acetylcholine in solutions containing hemicholinium-like substances. For example, when comparing the inhibitory activity of HC-3 and its *p*-terphenyl analogue TPHC-3 on acetylcholine biosynthesis by brain mince (Gardiner & Lee, 1969), the low acetylcholine content of some samples (4–10 nmol/g wt wet tissue) could not be detected on the usual eserinized rectus preparation in the presence of HC-3 or TPHC-3. Since ethanol is known to increase the sensitivity of the frog rectus abdominis to acetylcholine (Emmelin Nils, 1939), the following method of acetylcholine assay was adopted.

Modified Ringer solution of the following composition is used (g/litre): NaCl, 7·5; KCl, 0·15; $CaCl_2$, 0·2; $NaHPO_4$, 0·01; $NaHCO_3$, 0·2; glucose, 1; eserine salicylate, 0·01; and ethanol, 2% v/v; it is aerated continuously with 5% carbon dioxide in oxygen. One rectus abdominis muscle of the local toad (*Bufo melanostitus*) is suspended in a bath of 2 ml volume, and the preparation is used at room temperature.

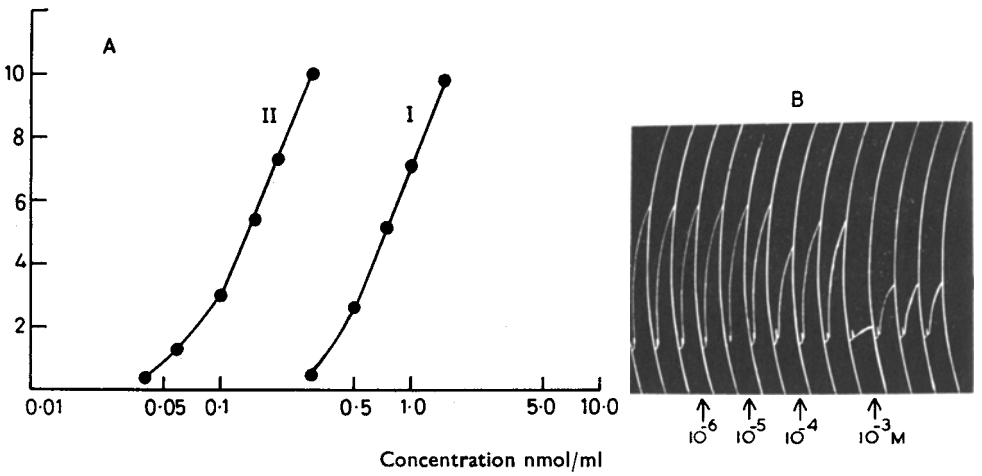

FIG. 1A. Concentration-effect curves of acetylcholine in 10^{-5}M HC-3. (I) In the modified Ringer solution without ethanol. (II) In the modified Ringer solution. Ordinate: recorded height of contraction (cm).

B. Contractions of a toad rectus abdominis muscle to 0·2 nmol/ml acetylcholine as recorded by a Gimbal lever having a magnification of 10 and exerting a 2·5 g tension on the muscle. Time cycle used was 3 min : 1 min exposure and 2 min rest period. At each arrow indicated HC-3 was added. HC-3 at concentration of 10^{-4}M and higher depressed the contractions.

Fig. 1A shows the presence of 2% ethanol in the Ringer, producing a useful increase in sensitivity of 5–6 fold.

The preparation remains stable for at least 4 h.

However, in the presence of higher concentration of HC-3 (10^{-4}M and above), the contractions of the muscle to acetylcholine are depressed (Fig. 1B), as reported by Bertolini & others (1967).

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Potentiation of [^{3}H]noradrenaline accumulation in rat heart by angiotensin

There have been many reports in recent years that angiotensin interacts with the sympathetic nervous system to potentiate sympathetic activity. Conflicting reports based on experiments made on isolated tissues *in vitro* and on isolated perfused organs indicate that angiotensin inhibits (Palaič & Khairallah, 1967; Panisset & Bourdois, 1968) or has no effect upon (Thoenen, Hürlimann & Haefely, 1965; Hertting & Suko, 1966) the uptake of noradrenaline at peripheral nerve endings. Results are also conflicting for *in vivo* experiments on the effect of angiotensin on myocardial uptake of noradrenaline. Thus Buckley (1965) found no significant alteration in rat myocardial catecholamines at the end of 1 h of angiotensin infusion, but several investigators (Westfall & Peach, 1965; Peach & Ford, 1968) found an early increase in myocardial noradrenaline in the intact rabbit and cat under the influence of angiotensin, although this increase was attributed to an increase in plasma noradrenaline due to angiotensin-induced release of noradrenaline from adrenal and peripheral nerve endings (Peach & Ford, 1968).

It would appear that studies on angiotensin-noradrenaline interaction in animals with an intact nervous system may offer some advantage over *in vitro* studies, because Zimmerman (1962) has shown that vasoconstrictor response to angiotensin in the perfused hindquarters of dog is partly dependent on an intact sympathetic innervation.

Fifty experiments were made on Charles River CD male rats of approximately 200 g weight. Rats were anaesthetized with sodium pentobarbitone, which has been shown not to influence the uptake of [^{3}H]noradrenaline (^{3}H-NA) in the cat (Whitby, Axelrod & Weil-Malherbe, 1961) and has been used with the same rationale in the guinea-pig (Crout, 1964). Twenty-five control rats were injected via left lateral tail vein with 0·9% saline and 25 rats were injected with 0·1 μg angiotensin II amide (Hypertensin Ciba) in 0·9% saline. One min after the injection, 40 μCi ^{3}H-NA (6·6–8·45 Ci/mmol, New England Nuclear) was injected over a period of 10 s into the right lateral tail vein. Rats were killed by cervical dislocation exactly 1 min after the second injection and the heart was quickly dissected, killing and dissection time being standardized to 25 s. The left ventricle of each heart was trimmed and washed twice with cold saline and blotted on Whatman No. 1 filter paper. Each specimen was weighed and then ground to a fine consistency with 0·4N perchloric acid and sea sand in a mortar. Specimens were washed twice and the material centrifuged and the supernatant brought to 10 ml volume. A 1 ml aliquot was added to 14 ml of counting solution and total radioactivity was counted in a Packard