shown that (−)-noradrenaline can cause excitation of brain stem neurons at the same pH as (+)-noradrenaline which does not cause excitation. Since (+)-noradrenaline has identical physicochemical properties to (−)-noradrenaline, the excitations cannot be explained simply on the basis of the pH effects which Frederickson & others (1971) suggest.

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The biological estimation of free choline in tissues

Chemical, microbiological and biological methods for the quantitative estimation of free choline in biological materials have been reviewed by Best & Lucas (1943) and Griffith & Nyc (1954). Only the biological method has been found to be reliable and of high sensivity and specificity for the estimation of free choline in plasma (Bligh, 1952). To adapt this method for estimating free choline in tissues, we conducted experiments to determine the recovery of choline added to rabbit tissue extracts.

Adult rabbits of either sex (1·3–1·6 kg) were anaesthetized with sodium pentobarbitone (50 mg/kg, i.v.) and decapitated. The whole brain, heart, lungs, kidney, liver (about 6 g), skeletal muscle (about 16 g) from the thigh region and small intestine (about 60 cm from the terminal end, after discarding the last 10 cm) were immediately removed and placed in ice-cold saline and kept at 0–4°. The lumen of the intestine was flushed with 0·9% sodium chloride solution (saline).

Each tissue was cleared of extraneous connective tissue, washed twice with ice-cold saline and dried with filter paper. The tissue was cut into two approximately equal parts which were quickly weighed; each part was cut into pieces and placed in a flask. 12% trichloroacetic acid solution (TCA) and distilled water were added (2 ml of each/1g tissue) to the control sample. To the test sample, instead of distilled water, an equal volume of choline chloride solution was added so as to give a final concentration of 200 (for muscle), 500 (for heart), 1000 (for brain) and 2000 nmol/ml tissue extract (for lung, liver, intestine and kidney). The tissue was homogenized with a blender for about 3 min, allowed to stand for 10 min and the mixture filtered through filter paper.

About 2 ml of the filtrate was collected in a 5 ml stoppered test tube and extracted 5 or 6 times with about one-third its volume of ether to remove excess TCA. The ethereal layer was discarded and the free choline content in 0·5 ml of the ether-extracted filtrate determined by the acetylation procedure of Gardiner & Domer (1968). The

acetylated product was bioassayed for acetylcholine on the longitudinal muscle strip of the guinea-pig ileum (Gwee & Lim, 1972).

Recovery of choline added to rabbit tissue extracts was expressed as (choline concentration of tissue extract containing choline chloride — choline concentration of control tissue extract)/concentration of choline chloride added X 100. Values were taken as the mean ± s.e. of four experiments. Recovery from all tissues amounted to 92–106% of choline added to the tissue extracts. The recovery (mean ± s.e. %) from the lung (96 ± 7), liver (93 ± 8), and intestine (92 ± 8) appeared to be slightly lower than from muscle (106 ± 8), heart (106 ± 8), brain (100 ± 10) or kidney (102 ± 4).

In preliminary experiments the ether-extracted filtrates (control), when tested on the longitudinal muscle preparation just before acetylation with acetyl chloride, showed no appreciable activity except those from intestinal segments, which had stimulant activity equivalent to about 20% of the acetylated product. This pre-acetylation activity was abolished by the antihistamine mepyramine (10 nmol/ml) added to the bath. All intestinal samples were therefore assayed in the presence of mepyramine. However, the drastic treatment with acetyl chloride also eliminates the activity of any histamine or, indeed, of any other pharmacologically active substances. The activity from all acetylated samples was abolished by atropine (10 nmol/ml).

The quantitative recovery of choline added to rabbit tissue extracts shows the method to be essentially free from interfering substances that affect the chemical methods of estimation. However, it does not distinguish choline from acetylcholine and, in tissues containing both, the "choline" estimated will include both the biologically free choline and any acetylcholine present, though tissue cholinesterase can be inhibited to allow easy determination of the latter. In the present study, we found this to be unnecessary because the contribution from acetylcholine to the free choline measured was negligible.

The biological assay method for the estimation of free choline in plasma can easily be adapted for use with tissues, and is reliable, simple, sensitive and reasonably specific.

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