Noradrenaline effects and pH

A recent communication by Frederickson, Jordan & Phillis (1971) suggests that the excitatory responses of some central neurons to the microiontophoretic application of noradrenaline, which were first noted in the cat by Johnson, Roberts & Straughan (1969a), are due to the low pH of 3·5 or less of the noradrenaline solutions used in these experiments. Whilst agreeing with Frederickson & others that the validity of noradrenaline excitatory responses as genuine neuronal effects may be in doubt (Stone, 1971, 1972), I do not believe that pH plays a significant part in the production of these responses.

That altering the pH of noradrenaline solutions over a wide range did not affect the excitatory actions of noradrenaline has been stated by Johnson & others (1969b). This is confirmed in Fig. 1 which shows excitatory responses produced by iontophoresis from 0·2 M noradrenaline solutions of pH 4·0, 3·0 and 5·0 respectively of a neuron in the cerebral cortex of a rat anaesthetized with urethane. The noradrenaline was applied with currents of 60nA at intervals of 3 min to reduce tachyphylaxis (Johnson & others, 1969b). The excitations produced at these pH levels are similar; the slight reduction that can be detected is probably due to tachyphylaxis. This demonstration has been made on 12 neurons in 3 rats. In no case has a noradrenaline response been affected by pH.

Furthermore, Krnjević & Phillis (1963) have reported that pH effects only become significant if H^+ ions *alone* are ejected with currents of approximately 60nA.

One major point made by Frederickson & others (1971) is that NaCl solutions adjusted to low pH values by acid are not adequate as controls for pH artifacts due to the low mobility and H^+ releasing properties of ionized noradrenaline. Hence the role of pH has gone undetected. However, besides the experiments described above and those of Johnson & others (1969b), Boakes, Bradley & others (1971) have

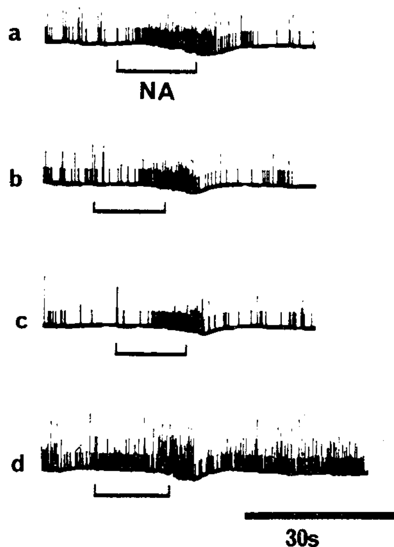

FIG. 1. The firing rate of a cell in the cerebral cortex of a rat anaesthetized with urethane is increased by the microiontophoretic application of noradrenaline with a current of 60nA (NA). The response is essentially the same when the noradrenaline solution is at pH 4·0 (a), 3·0 (b), or 5·0 (c). A slight reduction is probably due to tachyphylaxis. (d) shows that the excitation is still obtainable when the cell's firing rate is artificially increased by the iontophoresis of L-glutamate (30nA) (throughout record d).

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