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The excitation of neurons by noradrenaline

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Responses of single cortical neurons to microelectrophoretically applied noradrenaline at pH 3·1 and 5·0 and to hydrogen ions were compared in the halothane-anaesthetized cat. Of 16 neurons sensitive to noradrenaline, 13 were excited and 3 were depressed by noradrenaline at both pH values, whereas hydrogen ions released from an HCl solution did not affect the firing rate. Dose-response studies showed that noradrenaline at pH 3·1 was more potent than at pH 5·0. It is concluded that excitatory responses to noradrenaline are not artifacts and that the adjustment of the pH of noradrenaline solutions with NaOH should be avoided.

Evidence for the role of noradrenaline as a transmitter in the central nervous system has accumulated markedly in recent years. It now seems likely that noradrenaline is released from synaptic terminals and influences the activity of postsynaptic neurons. Investigations of its effects on single brain cells have usually involved the electrophoretic ejection of noradrenaline from multibarrelled micropipettes close to the neuronal soma. Using this technique several workers have reported both excitatory and inhibitory effects of noradrenaline (Yamamoto, 1967; Satinsky, 1967; Phillis & Tebēcis, 1967; Tebēcis, 1970; Boakes, Bradley & others, 1971; Hösli, Tebēcis & Schönwetter, 1971), whilst others have observed only inhibitory effects (Krnjević & Phillis, 1963; Bloom, Costa & Salmoiraghi, 1965; Engberg & Ryall, 1966).

Frederickson, Jordan & Phillis (1971) investigated the responses of cells in the neocortex of the cat to noradrenaline applied by electrophoresis from solutions at different pH values. They observed that at pH 4·0 noradrenaline was predominantly depressant, but at pH 3·0 and 3·5, 29 and 26% of the cells increased their firing rate. These authors suggested that this discrepancy could be explained in terms of an excitatory action of hydrogen ions released during the ejection of noradrenaline from solutions at acidic pH, a suggestion which they supported with a report that hydrogen ions ejected from solutions of hydrochloric acid could excite cortical neurons of decerebrate cats (Jordan, Lake & Phillis, 1972). These observations and conclusions are in direct contrast with those of Johnson, Roberts & others (1969), who reported that the direction (excitation or depression) of the responses of individual neurons did not depend on the pH of the solution within a range of 3·0 to 5·5. Similar results have been reported by Stone (1972) for rat neocortex.

We have re-examined the effects of noradrenaline applied to cortical neurons from solutions at different pH values, and also examined the effect of hydrogen ions ejected from hydrochloric acid solutions.

METHODS

Cats were anaesthetized with halothane and prepared for the study of single cells in the posterior sigmoid gyrus in the manner described previously (Roberts & Straughan, 1967; Bradshaw & Szabadi, 1972). Five-barrelled micropipettes with overall tip

diameters of 3–6 μm were filled with fresh drug solutions by centrifugation. Two barrels contained 4 M NaCl solution, one for the recording of action potentials, and the other for use in 'current balancing' (Roberts & Straughan, 1967). The remaining three barrels contained the following solutions: 0·2 M noradrenaline bitartrate (pH 3·1); 0·2 M noradrenaline bitartrate (pH 5·0); and 0·01 M hydrochloric acid (pH 2·0). A solution of noradrenaline bitartrate at pH 3·1 ($\pm$0·1) was obtained by dissolving (−)-noradrenaline bitartrate (Koch-Light) in glass-distilled water, whereas the solution at pH 5·0 ($\pm$0·1) was obtained by adjustment with 1 M NaOH solution. The pH values of these solutions were measured by a Pye Universal pH meter (model EJ 660). Hydrochloric acid solutions of 0·01 M were used because this was the lowest concentration which adequately carried electrophoretic currents.

The techniques used for the recording of action potentials, and for the electrophoretic application of drugs to neurons, have been described previously (Roberts & Straughan, 1967). For the purpose of constructing dose-response curves, the size of the neuronal response was expressed as the total number of spikes produced in response to the application of the drug (total spike number). The total spike number was calculated by measuring the total number of spikes generated between the onset of the drug application and the recovery of the base line firing rate, and subtracting the number of spikes emitted during an equivalent control period before the drug was applied. Spikes were counted via a Grass UI-1 unit integrator.

RESULTS

Only cells which satisfied the following criteria were considered suitable for study in these experiments: spontaneous firing, clearly isolated spikes of unchanging amplitude above 300 μV, stable baseline firing rate, insensitivity to current, sensitivity to noradrenaline and reproducibility of responses. After several minutes' recording of the baseline firing rate, the three drugs were applied repeatedly in a regular cycle for equivalent periods of time. A retaining current of −25 nA was applied between drug applications. The interval between successive applications of each drug was kept constant to enable us to make quantitative studies (Bradshaw, Roberts & Szabadi, 1973). This report is concerned with studies made on cells to which all three drugs were applied and which fully satisfied the above criteria throughout the study. The studies lasted on average 75 min, and occasionally up to 5 h. Sixteen studies were completed in 9 cats.

Comparison of noradrenaline at pH 3·1 and pH 5·0

Of the 16 cells studied, the 13 neurons that were excited by noradrenaline at pH 3·1 were also excited by noradrenaline at pH 5·0, and the 3 neurons depressed by noradrenaline at pH 3·1 were also depressed by it at pH 5·0. No cell responded differently to the drug at different pH values. An example of this finding is shown in Fig. 1. The latency and time-course of the responses to noradrenaline were similar to those reported previously (Johnson & others, 1969).

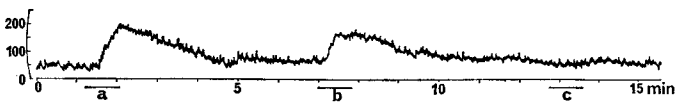

FIG. 1. Continuous recording of the firing rate of a single cortical neuron. Ordinate: firing rate (spikes s^{-1}); abscissa: time (min). Horizontal bars below the time base indicate drug applications. a: noradrenaline (pH 3·1), 100 nA; b: noradrenaline (pH 5·0), 100 nA; c: H^+, 100 nA. The cell was excited by noradrenaline pH 3·1 and pH 5·0, but failed to respond to H^+.

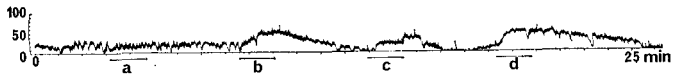

FIG. 2. Continuous ratemeter recording (as in Fig. 1). a: H^+, 75 nA; b: noradrenaline (pH 5·0), 75 nA; c: simultaneous application of noradrenaline (pH 5·0), 75 nA and H^+, 75 nA; d: noradrenaline (pH 5·0), 75 nA. The size of the excitatory response to noradrenaline (pH 5·0) was reduced when it and H^+ were applied simultaneously.

Effect of H^+

Of the 16 noradrenaline-sensitive cells, 15 did not respond to H^+, even when currents of up to 200 nA were used (see Figs 1 and 3). One neuron, which was depressed by noradrenaline, was also depressed by H^+. Changes in spike amplitude did not occur.

The application of H^+ frequently reduced the size of the response to subsequent application of noradrenaline. Furthermore, when noradrenaline (pH 5·0) and H^+ were ejected simultaneously from adjacent barrels of the micropipette, there was a reduction in the size of the excitatory response (Fig. 2).

Dose-response studies

It was observed on 9 cells that noradrenaline (pH 3·1) evoked a larger response than it did at pH 5·0 (see Fig. 1). As this suggested a difference in potency, we conducted comparative dose-response studies on three cells. A range of current intensities was used to compare the responses of the same cell to noradrenaline at pH 3·1 and at pH 5·0. The results obtained from one cell are displayed in Fig. 3 and the current-response curves derived from this study are shown in Fig. 4. At the lower current intensities both solutions were approximately equipotent, whereas when currents of higher intensities were used, noradrenaline at pH 3·1 was much more potent.

DISCUSSION

Our results confirm the observations of Johnson & others (1969) and Stone (1972) that noradrenaline ejected from solutions at high and low pH values has qualitatively the same effect. Thus we can conclude that the occurrence of excitatory responses to noradrenaline is not due to its use in solutions at low pH values. This conclusion is further supported by the frequent observation of excitatory responses in midbrain to noradrenaline applied from solutions at pH 5·0–6·0 (Boakes & others, 1971).

Furthermore, we were unable to confirm the findings of Jordan & others (1972) that cortical neurons can be excited by H^+ ejected from HCl solutions. Indeed, we have found that H^+ could reduce the size of the excitatory response to noradrenaline, when H^+ and noradrenaline were ejected simultaneously from adjacent barrels of the micropipette.

Although noradrenaline ejected from solutions at different pH values evoked qualitatively similar responses, the size of the depressant or excitatory responses of any neuron to noradrenaline ejected from a solution at pH 3·1 was usually greater than that of the response to noradrenaline ejected from a solution at pH 5·0. The dose-response studies show that when ejected from solutions at pH 3·1 noradrenaline was more potent than when ejected from solutions at pH 5·0, especially when electrophoretic currents of higher intensities were applied. The difference between the actions of noradrenaline released from solutions at different pH values was inter-

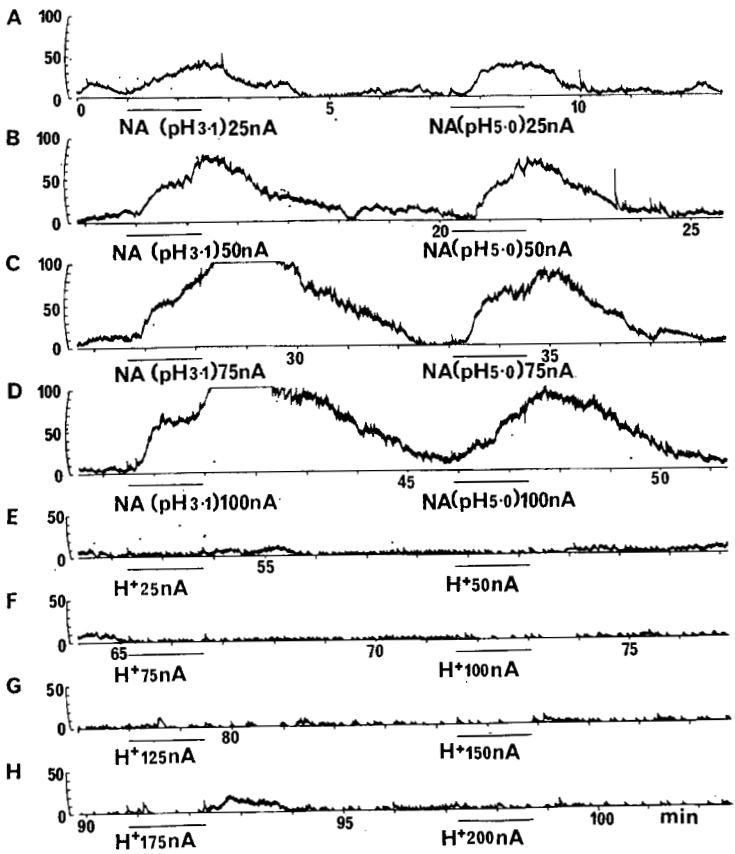

FIG. 3. Continuous ratemeter recording (as in Fig. 1). A–D: noradrenaline pH 3·1 and pH 5·0 applied with successively higher currents, evoked progressively larger responses. At higher current intensities noradrenaline pH 3·1 evoked larger responses than noradrenaline pH 5·0. E–H: H^+ (25–200 nA) failed to evoke responses.

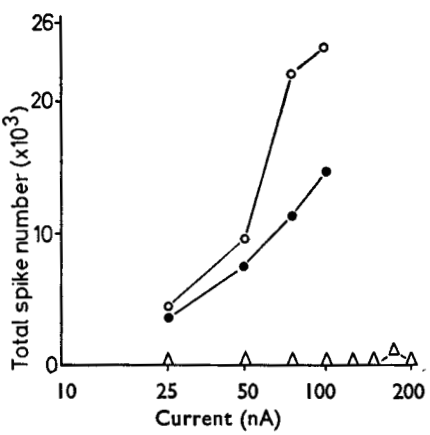

FIG. 4. Current-response curves for noradrenaline (pH 3·1) ○–○, (pH 5·0) ●–●, and for H^+ △–△ obtained from the data shown in Fig. 3. Total spike number (see Methods) is plotted against the intensity of the ejecting current (on a log scale). Noradrenaline at pH 3·1 is more potent than at pH 5·0 at higher current intensities.

preted by Frederickson & others (1971) in terms of an excitatory action of H^+ released from solutions at lower pH values. Such an explanation is obviously untenable. However, the apparent difference in potency may result from a lower rate of noradrenaline release from the solution at pH 5·0. This would be due to the large number of Na^+ and OH^- introduced into the solution when the pH was adjusted to 5·0 (0·1 M NaOH in the final solution). This would tend to reduce the transport number of noradrenaline (Bockris & Reddy, 1970). We have found in experiments *in vitro* that the addition of NaCl to the noradrenaline solution reduces the transport number of noradrenaline, and that the rate of release does not increase linearly with the intensity of the electrophoretic current. (At pH 3·1 the concentration of H^+ is in the order of 10^{-3} M, which, even taking the high absolute mobility of H^+ into account, would not have a significant effect on the transport number of noradrenaline).*

It is apparent, therefore, that there are no good reasons for avoiding the use of noradrenaline solutions at pH 3·1 in microelectrophoresis experiments. However, there are disadvantages associated with the adjustment of the pH to a higher level by the addition of NaOH: firstly, dose-response relations are distorted, and secondly, there is a greater risk of obtaining misleading observations due to the application of a biologically active product of oxidation.

We encounter excitatory responses to noradrenaline in the cerebral cortex regularly. The slow time-course of these responses (Johnson & others, 1969) is similar to that observed in other parts of the central nervous system (Yamamoto, 1967; Tebēcis, 1970; Boakes & others, 1971; Hösli & others, 1971). These responses can be selectively and reversibly antagonized by various α- and β-adrenoceptor antagonists (Johnson & others, 1969; York, 1970; Boakes & others, 1971; Bradshaw, Roberts & Szabadi, 1971b), and can be potentiated (Bradshaw, Roberts & Szabadi, 1971a; Avanzino, Ermirio & Zummo, 1971) or antagonized (Bradshaw, Roberts & Szabadi, 1971a) by tricyclic antidepressant drugs. As the excitatory response to noradrenaline shown in the figure published by Frederickson & others (1971) appears to be fundamentally different from the characteristically slow response reported by others, it would be of interest to know whether the excitatory responses observed by those authors are dose-dependent, and whether they can be antagonized and potentiated by the appropriate drugs.

We conclude that the excitatory responses observed by us are very likely to be genuine pharmacological responses of single neurons to noradrenaline, and are not artifacts produced by H^+.

* The transport number of noradrenaline (NA) (n) is expressed in the following equation:

$$n = \frac{k_1}{k_1 + k_2 + c_3u_3 + c_4u_4 + c_5u_5}$$

where c = equivalent concentration, u = absolute mobility; subscripts signify the ionic species: 1 = NA^+, 2 = tartrate, 3 = H^+, 4 = Na^+, 5 = OH^-; $k_1 = c_1u_1$ and $k_2 = c_2u_2$.

Substituting the equivalent concentrations and absolute mobilities of H^+, Na^+, and OH^-, the transport number of NA will be

$$\frac{k_1}{k_1 + k_2 + 0{\cdot}363} \text{ (pH 3·1) and } \frac{k_1}{k_1 + k_2 + 25{\cdot}7} \text{ (pH 5·0).}$$

It can be seen that the transport number of NA at pH 5·0 will be lower than the transport number of NA at pH 3·1.

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