Effect of pH on the release of noradrenaline from micropipettes

Frederickson, Jordan & Phillis (1971) have suggested that the excitatory responses of single cortical neurons to microelectrophoretically applied noradrenaline may be artifacts produced by the ejection of H^+ from acidic noradrenaline solutions. In contrast to Frederickson & others (1971), we have found (Bevan, Bradshaw & others, 1973) that the direction of responses to noradrenaline was not influenced by the pH of the solution. However, we observed that noradrenaline ejected from solutions at pH 3·1 usually appeared to be more potent than noradrenaline ejected from solutions at pH 5·0. We suggested that this difference was due to a lowering of the transport number of noradrenaline in the pH 5·0 solution produced by the addition of NaOH. We report here the results of some experiments which support this suggestion. In these experiments we measured the release of [^{14}C]noradrenaline from micropipettes *in vitro*.

Our methods for the preparation of the micropipettes and the collection of samples have been described elsewhere (Bradshaw, Roberts & Szabadi, 1973). Six-barrelled pipettes were used in these experiments. Three barrels contained noradrenaline-bitartrate at 0·2 M at pH 3·5; the remaining three contained it at 0·2 M at pH 5·0. The solution at pH 3·5 was made by dissolving the bitartrate in double distilled water and that at pH 5·0 by the addition of 1 N NaOH. The specific activity of noradrenaline bitartrate was 0·5 mCi mmol^{-1} in the final solutions. When the release from barrels containing one solution was being studied, spontaneous release from the other three barrels was either taken into account as a constant factor to be subtracted from the total release measured, or was eliminated by the passage of a high retaining current (−300 nA) which had proved instantaneously effective. 10 min sample collection periods were used.

Some of the characteristics of the five micropipettes used are summarized in Table 1. The electrical resistance was lower in the case of barrels containing noradrenaline at pH 5·0. The rate of spontaneous release was not influenced by the pH of the solution.

Electrophoretic release was measured using a range of ejecting currents (12·5–200 nA). With all the five micropipettes tested, any given ejecting current was less effective in releasing noradrenaline from the solution at pH 5·0 than from the solution at pH 3·5. The relation between current intensity and rate of electrophoretic release for one micropipette is shown in Fig. 1. Transport numbers were calculated for each micropipette (see Bradshaw & others, 1973). In every case the transport number of noradrenaline was lower when release was measured from the pH 5·0 solution (see Table 1).

The efficacy of retaining currents was studied in detail using only two micropipettes. The output of noradrenaline from the pH 5·0 solution was 2·6 times (micropipette No. 3) and 2·5 times (micropipette No. 5) greater than the output from the pH 3·5

Table 1. *Characteristics of micropipettes.*

Micropipette		Electrical resistance of each drug barrel (Mohm)						Rate of spontaneous release (pmol per barrel min^{-1}; mean ± s.e.)		Transport number (mean ± s.e.)	
	Tip diameter	NA (pH 3·5)			NA (5·0)						
Number	(μm)	1	2	3	4	5	6	NA (pH 3·5)	NA (pH 5·0)	NA (pH 3·5)	NA (pH 5·0)
1	8	60	60	60	25	25	25	24·12 ± 0·68	24·12 ± 0·68	0·18 ± 0·01	0·02 ± 0·00
2	4	75	75	100	50	50	50	2·65 ± 0·73	2·00 ± 0·68	0·21 ± 0·01	0·10 ± 0·00
3	4	65	65	65	30	30	30	3·40 ± 0·09	3·40 ± 0·09	0·28 ± 0·01	0·11 ± 0·00
4	4	95	95	95	60	60	60	4·62 ± 0·12	4·62 ± 0·12	0·36 ± 0·01	0·18 ± 0·01
5	5	85	85	85	40	40	40	9·07 ± 0·49	11·12 ± 0·40	0·43 ± 0·01	0·13 ± 0·01

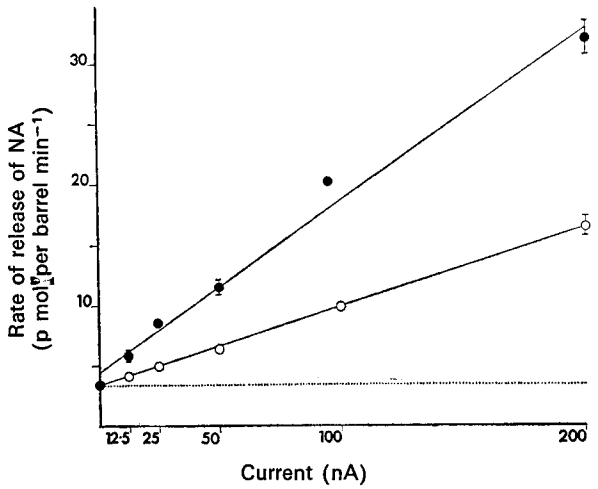

FIG. 1. Rate of release of noradrenaline (NA) at pH 3·5 (●——●) and pH 5·0 (○——○) from micropipette No. 3. Each point is the mean of four measurements. (Standard error is indicated except when it was less than ±0·25 pmol per barrel min^{-1}.) Both lines were obtained by linear regression. The rate of spontaneous release is indicated by the broken line.

solution during the application of −25 nA for a standard period (40 or 60 min). Thus both ejecting and retaining currents were less effective with the pH 5·0 solution.

The lower transport number of noradrenaline in the pH 5·0 solution is most likely to be due to the introduction of Na^+ into the solution when the pH was adjusted (Bevan & others, 1973). By titrating with 1 N NaOH a 0·2 M noradrenaline bitartrate solution, we have found that it behaves as a buffer within the pH range 2–5. This reflects the two pK_a values for tartaric acid (2·98 and 4·34; see Weast, 1972). Thus approximately 110 times more NaOH had to be added to the pH 3·5 solution in order to raise the pH to 5·0 than would have been predicted if only the H^+ existing at pH 3·5 had been titrated. Indeed, the concentration of Na^+ in the final solution at pH 5·0 was 0·1 M. The lowering of the transport number of noradrenaline in the pH 5·0 solution has been predicted on a theoretical basis previously (Bevan & others, 1973). It should be noted that OH^- should not have been included in the formula published in the footnote, since all added OH^- would associate with H^+ in a pH 5·0 solution. However, the present results demonstrate that this error does not invalidate the general prediction.

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