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Microiontophoretic release of drugs from micropipettes: use of ^{24}Na as a model

G. CLARKE, R. G. HILL AND M. A. SIMMONDS

Department of Pharmacology, The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX

Summary

1. The use of $^{24}Na^+$ of high specific activity allowed its iontophoretic release from multibarrelled glass micropipettes to be followed over short periods with low currents.

2. When a negative retaining current was passed to reduce diffusional efflux between the periods of positive current expulsion of $^{24}Na^+$, the rate of release of $^{24}Na^+$ during the expulsion period progressively increased during the first minute before becoming constant.

3. The currents employed were similar to those normally used to regulate the microiontophoretic release of potent drugs such as γ-aminobutyric acid. It is therefore concluded that, during the usual period of response to such drugs, the rate of release of drug is not constant but increasing.

4. The implications of these observations for the construction of microiontophoretic dose-response relationships is discussed.

Introduction

It is generally accepted that the microiontophoretic release of drugs from fine glass micropipettes (Curtis, 1964) obeys Faraday's law, i.e. the amount of drug released is proportional to the coulombs of charge passed. Supporting experimental evidence has been obtained by Krnjević, Mitchell & Szerb (1963) who showed that microiontophoretic release of acetylcholine was proportional to the charge passed down to 2·5 μcoulombs. More recent studies with radioactively labelled drugs have provided similar results for γ-aminobutyric acid (GABA), glutamate, noradrenaline and 5-hydroxytryptamine (Zieglgansberger, Herz & Teschemacher, 1969; Obata, Takeda & Shinozaki, 1970; Bradley & Candy, 1970; Hoffer, Neff & Siggins, 1971).

On the basis of this information, the rate of microiontophoretic release of a drug should be constant during the passage of a constant expelling current. However, when analysing the time course of the responses of single neurones to microiontophoretically applied GABA (Hill & Simmonds, 1973) we have found it necessary to postulate that the rate of microiontophoretic release of GABA at a low ejecting current (<40 nA) is not constant but rather increases with time during the period over which the response is obtained (i.e. the first minute). Since the total charge passed through the GABA barrel of the pipette to elicit a response in these experiments was generally less than the lowest charge passed in the direct measurements of release quoted above, it became necessary to extend the direct

measurements of release to lower charges. It was not possible to do this with GABA or any of the other putative neurotransmitter substances, however, since the radioactively labelled compounds were not available in sufficiently high specific activity to allow accurate measurement of the small amounts released. We, therefore, selected $^{24}Na^+$ as a model cation which was readily available in a high specific activity and we have studied its iontophoretic release from glass micropipettes, using low currents over short periods of time.

Methods

Seven barrelled glass micropipettes having an overall tip diameter of 5–7 μm were filled by centrifugation. Five of the outer barrels were filled with 0·9% w/v ^{24}NaCl solution of specific activity 1·2 mCi/ml (Radiochemical Centre, Amersham) and the remaining outer barrel contained 1 M NaCl. The centre barrel, which is usually used for recording, was left empty. At all times the total current passing through the five ^{24}NaCl barrels was balanced by an equal and opposite current through the 1 M NaCl barrel.

$^{24}Na^+$ was expelled simultaneously from five barrels into either 10 ml 0·9% NaCl or a 50 mg piece of cerebral cortex from a freshly killed rat. In both cases, the tip of the pipette was rinsed with saline and lowered into the medium. A negative retaining current was passed for 60 s through the ^{24}Na barrels and then the positive expelling current was passed for the required time. The passage of retaining and expelling currents was repeated and the pipette then withdrawn from the medium. Thus, each sample of medium contained ^{24}Na equivalent to that released from ten barrels during a single expulsion cycle.

The pieces of cerebral cortex were each dissolved in 0·5 ml Soluene (60° C for 60 min) and the volume made up to 10 ml with water. All samples were assayed for radioactivity by the Cerenkov method (Parker & Elrick, 1970) in a liquid scintillation counter at the settings used for tritium. All counts were corrected for efficiency and decay and the $^{24}Na^+$ content of each sample was expressed as picomoles Na^+ released/barrel during a single period of iontophoretic expulsion. Values in excess of 0·5 picomoles/barrel were all derived from a radioactive count which was at least double the background count.

The transport number (n) for Na^+, i.e. the fraction of current carried by Na^+, was calculated according to Faraday's law from the equation:

$$n = \frac{\text{picomoles } ^{24}Na^+ \text{ released}}{\mu \text{ coulombs passed} \times 10}$$

Results

Four similar pipettes were used in this study. In experiments with two of them 0·9% NaCl solution was used as the medium into which $^{24}Na^+$ was expelled, while in experiments with the other two pipettes both brain tissue and saline were used. There were no marked differences in the patterns of $^{24}Na^+$ release which could be attributed to the particular medium being used.

When $^{24}Na^+$ was expelled with a current of +20 nA from a retaining current, between expulsions, of −25 nA, all pipettes showed a progressively increasing rate of release of $^{24}Na^+$ over approximately the first 40–60 s (Figures 1 and 2). Thereafter, the rate of release became constant. If a calculated line representing the con-

stant rate of release between 40 and 120 s was projected to intersect the time axis at zero ^{24}Na release, the point of intersection in all cases had a value significantly greater than zero ($P<0.01$). This value was significantly reduced ($P<0.05$) in 3 out of 4 cases when the expelling current was increased from 20 nA to 40 nA, as in Figure 1. It was thus apparent that the period over which the rate of release of ^{24}Na was increasing became shorter with an increase in expelling current and a higher constant rate of release was achieved.

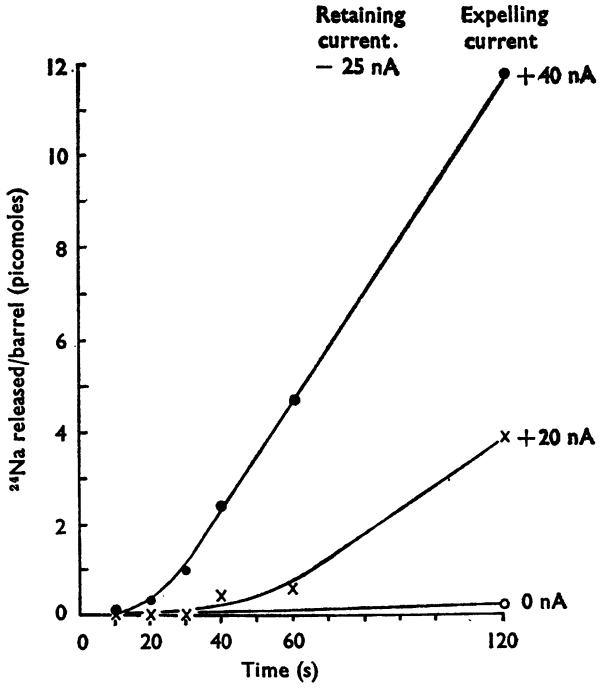

FIG. 1. Microiontophoretic release of $^{24}Na^+$ into rat cerebral cortex *in vitro*. Each point is the mean release/barrel from 5 barrels of a glass micropipette due to the passage of +40 nA (●), +20 nA (×) or 0 nA (○) through each barrel for the time shown. Before each period of release, a retaining current of −25 nA was passed through each barrel for 60 seconds. There was no detectable release of $^{24}Na^+$ during the passage of retaining current.

With two pipettes, the effect of reducing the retaining current was investigated. $^{24}Na^+$ was expelled with a current of +20 nA from a retaining current of either −25 nA or −5 nA. With each pipette, the lower retaining current caused a significant shortening ($P<0.05$) of the period over which the rate of release of $^{24}Na^+$ was increasing but the final constant rate of release was not affected by the size of the retaining current (Figure 2). Although the primary purpose of these experiments was to determine the effects of changes in retaining and expelling currents on the shape of the ^{24}Na release curve, the data obtained also allow estimates of the transport number for ^{24}Na to be made. In the example shown in Fig. 1, the constant rate of release between 60 and 120 s was approximately twice as large at 40 nA as at 20 nA giving transport numbers for $^{24}Na^+$ of 0·30 and 0·27 respectively. With two of the pipettes, however, the overall rate of release of $^{24}Na^+$ was markedly higher throughout (e.g. Fig. 2) and the constant rate of release at 40 nA was only fractionally higher than at 20 nA. With these pipettes, the apparent transport

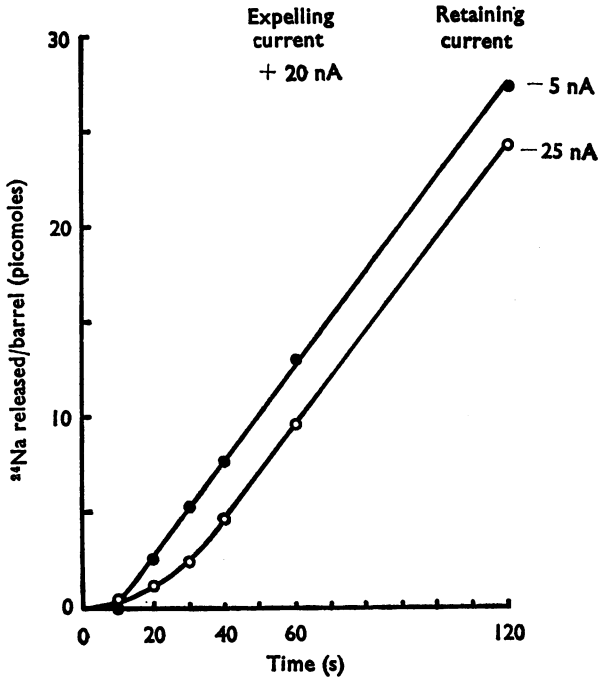

FIG. 2. Microiontophoretic release of $^{24}Na^+$ into 0·9% NaCl solution. Each point is the mean release/barrel from 5 barrels of a glass micropipette due to the passage of +20 nA through each barrel for the time shown. Before each period of release, a retaining current of −25 nA (○) or −5 nA (●) was passed through each barrel for 60 seconds. With this pipette, measurable amounts of $^{24}Na^+$ diffused from the tip during the retaining period at both currents.

numbers for $^{24}Na^+$ were impossibly high (greater than 1) indicating that a substantial proportion of the release was due to diffusion, as distinct from iontophoresis. A meaningful estimate of the transport number for $^{24}Na^+$ could be obtained in these cases, however, by taking the difference between the constant rates of release at 40 nA and 20 nA. The transport numbers did not depend on whether the medium used was brain tissue or saline, the values being $0{\cdot}36 \pm 0{\cdot}06$ and $0{\cdot}37 \pm 0{\cdot}04$ respectively (mean ± S.E.M. of 4 values in each case).

Discussion

During an iontophoretic expulsion of $^{24}Na^+$ from a micropipette, the progressive increase in rate of release of $^{24}Na^+$ during the initial period appears to be dependent upon the size of the prior retaining current. The larger the retaining current, the greater is the period over which the rate of release increases before becoming constant. The period of increasing rate of release can, however, be shortened by applying a higher expelling current.

These results can be predicted from a consideration of the rationale for using a retaining current (del Castillo & Katz, 1957). If zero current is passed through a drug-containing barrel of a micropipette, some drug will diffuse out and this may be adequate to produce a biological response. A retaining current of opposite charge to the drug is, therefore, passed to reduce the diffusional efflux. This is presumably achieved by the drug in the tip of the pipette being diluted with other

ions drawn in from the medium during the passage of the retaining current. Upon reversal of the current to expel the drug, a substantial part of the current will be carried initially by those ions previously drawn in from the medium. Only as these are lost will the concentration of drug inside the tip return to normal. Thus, both the transport number of the drug and its diffusional efflux will increase during the initial period of release, the duration of this period depending on the size of the previous retaining current and the length of time for which it was applied.

Since this explanation of the results is independent of the particular ions involved, $^{24}Na^+$ is a valid model for ionized drugs. Previous measurements of the microiontophoretic release of certain drugs themselves, however, failed to reveal an initial increase in the rate of release of drug (Krnjević *et al.*, 1963 ; Zieglgansberger *et al.*, 1969 ; Obata *et al.*, 1970 ; Bradley & Candy, 1970 ; Hoffer *et al.*, 1971). This was due to the fact that the first measurement was generally taken at 1 min or later when the rate of release might have already become constant. Nevertheless, if certain of the published release curves (see Hoffer *et al.*, 1971) are projected through their origins, an initial increase in rate of release does become apparent.

The practical importance of the present results is in relation to the microiontophoretic application of highly potent drugs. Such drugs normally require to be restrained from diffusing out of the tip of the pipette by passage of a retaining current and, when they are expelled, they produce a response quite rapidly. It is clear from Fig. 1 that, under these circumstances, the amount of drug released during a fixed period of expulsion bears no simple relationship to the current passed. As a consequence, the expelling current is not a good index of the ' dose ' of drug applied. This discrepancy is even greater in pipettes with a high diffusional component of release (e.g. Fig. 2) since diffusion depends only on the concentration of drug within the tip of the pipette and not on expelling current.

A more useful alternative would be to relate the ' dose ' of drug applied to the duration of its release at constant current since, during a single microiontophoretic application, the ' dose ' of drug applied progressively increases with time. Although this relationship between ' dose ' and time is not linear during the usual response period, it may, in practice, be sufficiently close to an exponential that time can be regarded as proportional to log ' dose '. Thus, during a single microiontophoretic application of a drug, the plot of response versus duration of application may be taken to represent the conventional response versus log ' dose ' relationship (Hill & Simmonds, 1973). To obtain reproducible responses, however, it is essential that the size and duration of the retaining current passed between periods of drug release are both kept constant.

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