Brit. J. Pharmacol. (1963), **20**, 491–496.

IONTOPHORETIC RELEASE OF ADRENALINE, NORADRENALINE AND 5-HYDROXYTRYPTAMINE FROM MICROPIPETTES

BY

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(*Received March* 10, 1963)

Estimations have been made of the amounts of adrenaline, noradrenaline and 5-hydroxytryptamine released by iontophoresis from micropipettes. In most experiments, the amount released was linearly related to the total electrical charge, but the transport numbers for different pipettes, filled with samples of the same solutions of adrenaline or of noradrenaline, varied substantially. Two pipettes containing noradrenaline failed to release any appreciable amounts of the drug. The transport number of 5-hydroxytryptamine was relatively constant (mean, 0.14).

The iontophoretic method of applying sympathomimetic drugs to neurones in the central nervous system is being used more and more extensively (Curtis & Davis, 1962 ; Bradley & Wolstencroft, 1962 ; Krnjević & Phillis, 1963a, b). It is therefore of interest to know how much drug is released from a micropipette by iontophoresis. Studies, made with pipettes containing acetylcholine chloride, have shown that the average fraction of the total current carried by ionized acetylcholine (its transport number) in such pipettes is not very different from that observed with a large volume of solution, but there was a substantial variation in behaviour between one pipette and another (Krnjević, Mitchell & Szerb, 1963).

It is preferable to study the iontophoretic release of drugs by currents which are at least similar in magnitude to those used in experiments on nerve cells. Inevitably only very small amounts of drugs are thus available for estimation. We have now examined the iontophoresis of adrenaline, noradrenaline and 5-hydroxytryptamine, for which adequately sensitive methods of assay were available.

METHODS

The micropipettes consisted of five Pyrex glass tubes sealed together and pulled out to a fine tip, as described by Krnjević & Phillis (1963a). They were similar to those used in previous studies of cerebral cortical neurones (Krnjević & Phillis, 1963a, b). The compound tips had outside diameters of 6 to 12 μ.

In the present experiments, only one of the barrels was filled with a concentrated solution of a drug, the others containing merely water. The solutions were prepared 48 hr before the experiments to allow time for diffusion to the tips. The concentrations of drugs were as

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follows: 0.13 M-5-hydroxytryptamine creatinine sulphate (May & Baker); 0.75 M-adrenaline acid tartrate (British Drug Houses); and 1.7 M-noradrenaline (British Drug Houses) acidified with concentrated hydrochloric acid. All three solutions had a *p*H between 3 and 4.

The pipettes were held vertically, with the tip dipping in 1.0 ml. of 150 mM-NaCl, to which was added either a trace of ascorbic acid (about 10 mg/100 ml.) to preserve 5-hydroxytryptamine, or hydrochloric acid (giving a final concentration of 2 mM) to preserve the catechol amines.

The iontophoretic currents were measured by a series galvanometer, with an accuracy of 1 nA (10^{-9} A), and there was a 50 MΩ resistor in series with the micropipette to reduce fluctuations in the current. The latter was kept at 100 nA in all experiments, well within the range of currents used when testing nerve cells. Four to six different periods of release (lasting up to 10 min) were tried with each micropipette, so that the relation between the release and the total electrical charge could be examined over a substantial range of times. Samples of fluid were also collected during periods when the drugs were allowed to diffuse out spontaneously, to estimate the magnitude of any spontaneous leak.

All samples were assayed within 2 to 3 hr and, for controls, several solutions of known concentration were handled in the same way to test for any possible loss of drug or contamination during handling; none could be detected.

Estimations. Noradrenaline and adrenaline were estimated fluorimetrically as described by Sharman, Vanov & Vogt (1962), but a Locarte filter fluorimeter was used. For noradrenaline, the primary filter was an LF/2 and the secondary an Ilford No. 625; for adrenaline the primary filter was a Corning 3880 with a half standard thickness Corning 3113, and the secondary a Chance OY4. The accuracies of this method are about $\pm 10\%$ in the upper range of concentrations for noradrenaline and about $\pm 5\%$ for adrenaline. The lower limits of accurate estimation are about 50 and 20 pmole for noradrenaline and adrenaline respectively. Hence, the lowest values in our results are only very approximate, being liable to errors approaching $\pm 100\%$.

5-Hydroxytryptamine was estimated by its fluorescence in 3 N-HCl (Udenfriend, Bogdanski & Weissbach, 1955) using an Aminco-Bowman spectrofluorimeter. The lower limit of estimation was about 10 pmole.

RESULTS

In all, twelve multibarrelled micropipettes were used, four containing adrenaline, four noradrenaline and four 5-hydroxytryptamine solution.

The amount of iontophoretic release of drug was plotted against the electrical charge after making due correction for any spontaneous leakage of drug. The general relation between the release of drug and the charge was obtained from the slopes of lines drawn through the experimental points. Examples of such graphs are shown in Figs. 1 and 2. The effective transport number was calculated by multiplying the slope (expressed in mole/coulomb) by 96,500.

Adrenaline

The values of the transport numbers were 0.13, 0.14, 0.20 and 0.37 (mean, 0.21). The full range of slopes is illustrated by the lines in Fig. 1. It can be seen that there was relatively little scatter of points obtained with any one pipette, and that all the lines, when extrapolated, went through or near the origin.

The marked difference between the slopes was associated with corresponding variations in tip diameter, electrical resistance and, especially, in the amount of

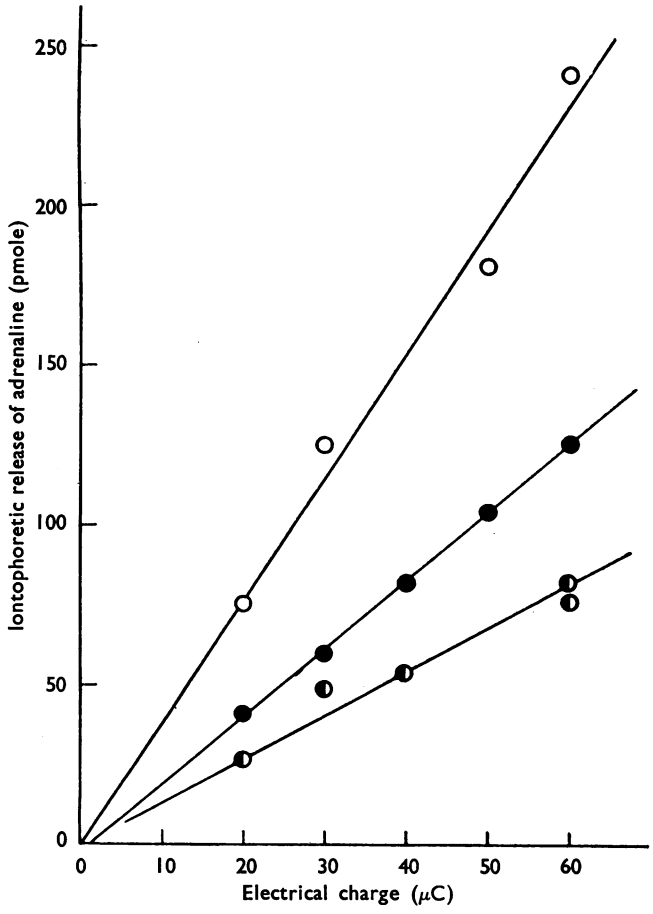

Fig. 1. The relationship between iontophoretic release of adrenaline (ordinate, pmole) and electrical charge (abscissa, μcoulomb) for three different micropipettes containing adrenaline acid tartrate. The current was 100 nA. Straight lines were positioned by eye.

spontaneous leak of adrenaline. Thus the smallest rate of release was from a pipette with a 7 μ tip (overall outside diameter) and a resistance much greater than 100 MΩ, and with a spontaneous leak of only 0.3 pmole/min; whereas the steepest slope was given by a 12 μ diameter pipette, with a tip resistance of 70 MΩ and a leak of 25 pmole/min.

Noradrenaline

Two micropipettes allowed current to flow without any difficulty; the values of the transport numbers were 0.34 and 0.37 and the spontaneous leakage was 0.5 to 1 pmole/min. The experimental points obtained with one of these pipettes are shown in Fig. 2 (o—o).

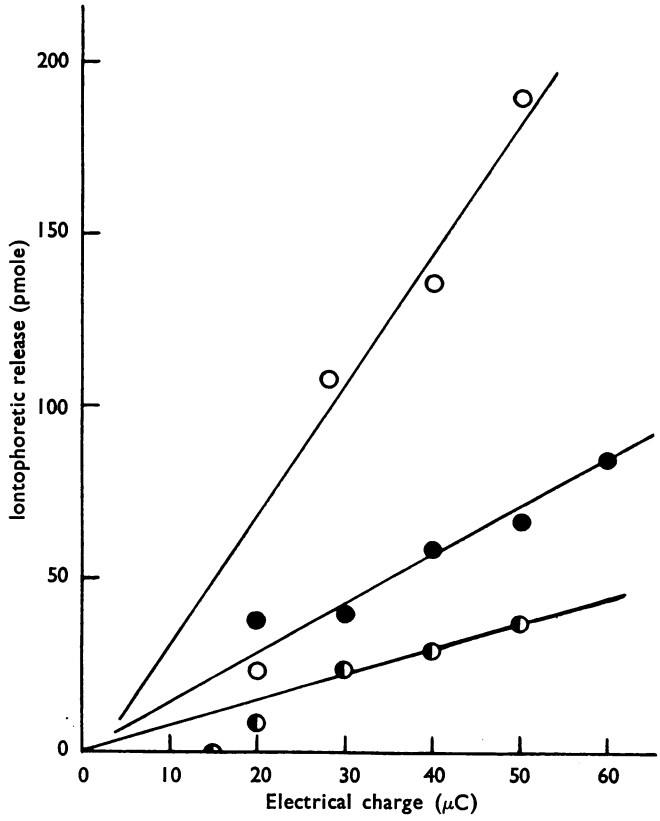

Fig. 2. The relationship between iontophoretic release (ordinate, pmole) of noradrenaline and of 5-hydroxytryptamine and electrical charge (abscissa, μcoulomb). ○——○ and ◐——◐: release of noradrenaline from two micropipettes containing noradrenaline hydrochloride. ●——●: release of 5-hydroxytryptamine from another pipette containing 5-hydroxytryptamine creatinine sulphate. The iontophoretic current was 100 nA in all instances. Straight lines were positioned by eye.

The other two pipettes did not behave so satisfactorily: current did not flow regularly because of a large and variable increase in the tip resistance. Hence much larger voltages had to be applied to keep the current flow at 100 nA (about 100 V instead of the usual 10 V). Moreover, no spontaneous leak of drug could be detected, so any leak was probably less than 0.1 pmole/min. These two pipettes did not release noradrenaline in a way clearly related to the iontophoretic current. One of them was tested on two different occasions: the first time there was only a minimal release of noradrenaline, which apparently did not vary with the amount of charge; on the second occasion the release apparently increased somewhat with the charge (points ◐—◐ in Fig. 2). However, as all these estimates were at the lower limit of sensitivity of the method, it is not certain that there was any significant release of noradrenaline. The transport number was probably in the range of 0.0 to 0.07. The other pipette giving only a doubtful release of noradrenaline gave a transport

number in the range 0.0 to 0.02 ; but, after several applications of a high voltage (with reversal of the polarity from time to time), the same pipette appeared to conduct a current more readily, and the value of the transport number rose to 0.17 (this same procedure applied to the other pipette did not change its behaviour). In this instance there was no obvious correlation between tip diameter or resistance and the transport number.

5-Hydroxytryptamine

The four pipettes containing the 5-hydroxytryptamine solution behaved in the most consistent manner. A typical result is indicated in Fig. 2 (●—●). The four values of the transport numbers were 0.10, 0.13, 0.14 and 0.18 (mean, 0.14). The spontaneous leak of drug was usually 1 to 2 pmole/min.

DISCUSSION

The most significant result of the present experiments is the demonstration of substantial variations in the effective transport number determined when a similar current flows through samples of the same solution of a drug in different micropipettes, even though the latter were prepared at the same time and from similar glass tubing. This result was shown, for instance, by the variations in the release of adrenaline from the pipettes containing solutions of adrenaline acid tartrate.

The mean value of the transport numbers for adrenaline was 0.21, which is only about two-thirds of that for the release of acetylcholine from solutions of acetylcholine chloride (Krnjević *et al.*, 1963). The lower mean value and the scatter of results with adrenaline may be due to variations in ionization of the acid tartrate in the tip of the pipette.

The micropipettes containing 5-hydroxytryptamine showed least variation in transport numbers, which were consistently relatively low. This result supports the suggestion that the creatinine component in solutions of 5-hydroxytryptamine creatinine sulphate probably carries one-half of the iontophoretic current (Curtis & Davis, 1962). However, it does not seem safe to assume on this basis that 5-hydroxytryptamine has double the potency of other drugs, as these (such as adrenaline) may have a similar transport number.

The experiments with noradrenaline presented a special problem, since the micropipettes either released a relatively large amount of drug or practically none. Use of such pipettes giving extremely low values of transport number could be very misleading when testing nerve cells since, although the micropipettes would be conducting the appropriate current, hardly any noradrenaline would leave their tips. It must be supposed that under these conditions the tip behaves as a semipermeable membrane (perhaps because it is blocked by some contaminating particle) which allows the passage of hydrogen or chloride ions but not those of noradrenaline.

Evidently one cannot assume that iontophoresis is a strictly quantitative method of application of drugs when comparing different compounds, and it is clearly essential to make observations with several pipettes before drawing any general conclusions about the effectiveness of any drug.

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