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COMPARISON BETWEEN FLUORIMETRIC AND RADIOCHEMICAL ASSAYS OF IONTOPHORETICALLY RELEASED NORADRENALINE FROM A SEVEN-BARRELED MICROPIPETTE

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Microiontophoretic methods enable the study of actions of putative chemical transmitters and central acting drugs on neuronal activity in the central nervous system. Before attempting such neuropharmacological experiments, however, the definite amount of the chemical substance released from a micropipette has to be accurately determined. In the present work, we determined the amount of noradrenaline (NA) released from a seven-barreled micropipette, using a fluorimetric method, and compared the findings with those seen in radiochemical assays.

Aqueous solution containing DL-NA-1-1'-³H with specific activity of 30 Ci/m mole

(Commissariat a l'Energie Atomique) was freeze-dried and then dissolved in 250 μ l of 0.5 M DL-NA hydrochloride solution as described by Hoffer et al. (1). The specific radio-activity of 1 pmol of this NA solution was approx. 80 disintegrations per min. A seven-barreled micropipette was filled with 3 M NaCl, and one of the outer barrels was replaced by a solution of 0.5 M unlabelled NA (pH 4.5) or tritiated NA, using the conventional technique. The electrode tip was manually broken in a manner done for physiological experiments, and then placed vertically into 0.5 ml of Ringer's solution.

NA in the micropipette was released by a current up to 200 nA delivered from a Micro-iontophoresis Programmer (Model 160, W-P Instruments). The amount of unlabelled NA in the Ringer's solution was microassayed by the modified trihydroxyindole method described by Von Euler and Floding (2), and Anton and Sayre (3). Modification was as follows; the reaction was suspended by adding 0.2 ml of solution containing 2 mg/ml of ascorbic acid in 5 N NaOH 2 min after adding 0.02 ml of 0.25% potassium ferricyanate to 0.5 ml of the sample. This method enabled the determination of as little as 1.4 ng/ml of

FIG. 1. Relationship between the amount of noradrenaline (NA) released and the ejection current or ejection time. A and B: The amount of NA released against the ejection current for 4 min. C and D: The amount of NA released against the ejection time (min) passed at 100 nA. A and C: Radiochemical assay, and B and D: Fluorimetric assay.

NA in standard solution. Tritiated NA released was transferred into a scintillation vial and a total of 10 ml of scintillator was added to each sample. The scintillator contained 0.4% 2,5-diphenyloxazole and 0.01% 1,4-di-2-(5-phenyloxazolyl)-benzene in toluene with 33% Triton x-100. The radioactivity was counted using a liquid scintillation spectrometer (Packard Tris-Carb). The transport number was calculated from the formula: $n = MZF/IT$, described by Curtis (4). Where, M = Moles of drug ejected, Z = Equivalence/mol, F = Faraday's constant, I = Ejection current (ampere), and T = Ejection time (sec).

Fig. 1A and B demonstrates the relationship between the amount of NA released and the current ejected for 4 min. The mean rates of spontaneous release of NA from the micropipette were 3.6 ± 1.0 (S.E.) and 3.6 ± 0.8 pmole/min with the radiochemical and fluorimetric assays, respectively. A linear relationship between the amount of NA and the ejection current was obtained in both measurements, and the correlation coefficients ($P < 0.01$) were 0.811 and 0.827 in the radiochemical and fluorimetric measurements, respectively. A similar linearity was observed between the amount of NA and ejection time at 100 nA (Fig. 1C and D). The correlation coefficients ($P < 0.05$) were 0.709 and 0.667 on the radiochemical and fluorimetric measurements, respectively. The mean transport number of each pipette was calculated at the ejection current of 50, 100 and 200 nA for 2 min. It was 0.23 ± 0.04 (S.E.) in the radiochemical assays, and 0.15 ± 0.02 in the fluorimetric assay (Table 1).

If hydrodynamic outflow of the solution and other factors are disregarded, the rate of spontaneous NA release is theoretically proportional to the concentration of NA in the pipette and square of the tip radius, according to Fick's first law of diffusion. However, it has been demonstrated that the rate of spontaneous release of NA was mainly proportional to the NA concentration rather than the tip radius (5). Therefore, the relatively high rate of NA release in the present experiment is considered to be due to the high concentration of NA in the pipette. The rate and amount of NA obtained herein was comparable to those reported by Hoffer et al. (1), who used the same concentration (0.5 M). The value of NA release which was dependent upon the ejection current by the fluorimetric assay, was also comparable to that reported by Krejvic et al. (6). In the present study, the transport

TABLE 1. Transport numbers in the radiochemical and fluorimetric measurements

Electrode No.	Radiochemical assay	Fluorimetric assay
1	0.115*	0.116
2	0.199	0.151
3	0.255	0.193
4	0.346	
Mean	0.229	0.153
S.E.	± 0.049	± 0.022

*: Transport number of each pipette was calculated at the current of 50, 100 and 200 nA ejected for 2 min, and then the mean of each pipette was obtained.

number measured by radiochemical assay was larger than that measured by the fluorimetric assay, and such may occur if some of radioactivity released is not associated with NA *per se* (7). Transport numbers reported by other investigators (1, 6, 8-10) are variable in the range of 0.05 to 0.37 and such is attributed to differences in experimental conditions. The transport number obtained herein in both measurements is within the range of these same values. Thus, a linear relationship between the amount of NA release and the ejection time or ejection current was obtained in both radiochemical and fluorimetric measurements. The latter method is simpler and more convenient than the former, because the same micro-electrode, from which NA release was checked in the latter assay, can also be used for electrophysiological experiments.

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EFFECTS OF DIFENAMIZOLE ON CONTENT OF CATECHOLAMINES AND METABOLITES IN MOUSE BRAIN

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In earlier studies in mice, a favorable correlation was found between the analgesic activity and cerebral concentration of 1,3-diphenyl-5-(2-dimethylaminopropionamide)-pyrazole[difenamizole, DFZ] (1). In a subsequent study, Nabeshima and Kameyama