

Short communications

Uptake of dopamine by platelets *in vivo*D. J. BOULLIN, E. M. McMAHON*
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Circulating platelets of cats infused with ^{14}C -dopamine take up the amine against a concentration gradient, with maximal concentration occurring 10 min after infusion. 58% of the platelet-bound radioactive dopamine was localized in a subcellular fraction rich in 5-hydroxytryptamine (5-HT) storage organelles. The results support earlier work with human platelets showing *in vitro* dopamine uptake by normal platelets, and *in vivo* accumulation in parkinsonian patients treated with L-dopa.

We have described the *in vitro* uptake of dopamine by normal human platelets and cells from both untreated and L-dopa-treated parkinsonian patients. This phenomenon seems to occur *in vivo* in man,

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since we found a dopamine-like substance in the platelets of the patients with Parkinson's disease who received oral L-dopa (Boullin & O'Brien, 1970). We have now explored *in vivo* uptake of dopamine further by infusing ^{14}C -dopamine into cats.

Methods.—Four male cats (2.3–3.3 kg) were anaesthetized with 35 mg/kg sodium pentobarbitone intraperitoneally. ^{14}C -dopamine (211 ± 1.3 nmol/kg) was dissolved in Krebs solution containing 0.6 mg/ml ascorbic acid and infused into the right femoral vein at a constant rate (2 ml/min for 2 min) with an infusion pump. Blood was collected from the right carotid artery through a polyethylene cannula. Other experimental details are given in the earlier paper (Boullin & O'Brien, 1970). Dopamine was separated from metabolites by the method of Robinson, Lovenberg, Keiser & Sjoerdsma (1968).

Results.—One min after the completion of the 2 min dopamine infusion, only 15% of the injected radioactivity remained in the plasma, equivalent to 86.8 ± 8.4 nmol. This was made up of 57 nmol of metabolites and 30 nmol unmetabolized dopamine. At this time the platelets had accumulated considerable amounts of dopamine (Fig. 1); thus the total number

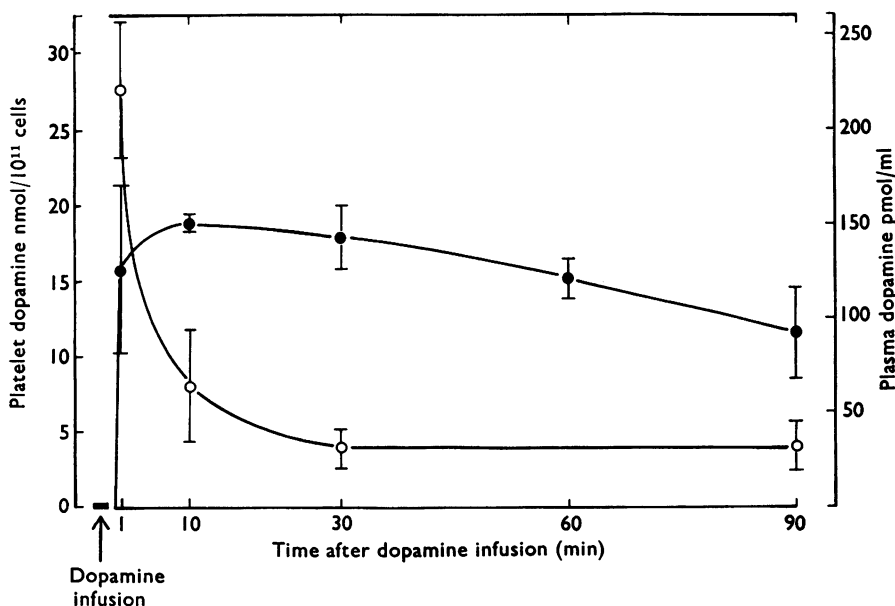


FIG. 1. Distribution of ^{14}C -dopamine in platelets (●) and plasma (○) after intravenous infusion of $0.21 \mu\text{mol/kg}$ over 2 min into the femoral vein. The figure shows platelet dopamine (nmol/10¹¹ cells, left ordinate) and plasma dopamine (pmol/ml, right ordinate) plotted against time after dopamine infusion (abscissa). Values are the mean \pm standard error of the mean (S.E.M.) of observations made in four cats.

of platelets (0.7×10^{11} cells) contained 11.1 ± 0.8 nmol, or $37 \pm 3\%$ of the dopamine in the circulation. Plasma dopamine declined rapidly after 1 min (Fig. 1) but was constant from 30 to 90 min. Thus, the proportion of circulating dopamine present in the platelets gradually increased, reaching a maximum of 75% 30 min after infusion.

This *in vivo* dopamine uptake was also compared with *in vitro* uptake by taking blood samples before dopamine infusion and then incubating either platelet-rich plasma or whole blood with ^{14}C -dopamine in a metabolic incubator for 10 to 90 min. We found that *in vitro* uptake of amine by platelets suspended in whole blood for 90 min (72 nmol/ 10^{11} cells) was considerably greater than that occurring in the circulation (12 nmol/ 10^{11} cells) (Fig. 1). On the other hand, *in vitro* uptake in blood was comparable to that observed with platelets suspended in plasma (93.8 ± 13.4 nmol/ 10^{11} platelets, four experiments).

The subcellular distribution of dopamine in platelets after *in vivo* labelling was studied using the differential ultracentrifugation method of Maynert & Isaac (1968). We worked with platelets removed 90 min after dopamine infusion, and then disrupted by sonification. After low speed centrifugation of the disrupted material ($2,000 g \times 20$ min), the platelet radioactivity was approximately equally distributed between the supernatant and particulate fractions. When the supernatant fraction containing platelet granules was centrifuged at high speed ($20,000 g \times 30$ min) to sediment these granules, 60% of the radioactivity was present in the pellet. The pellet was then resuspended in 0.5 ml of 0.44 M sucrose + EDTA and an aliquot layered onto a continuous sucrose gradient (1.0–2.0 M) and centrifuged at $130,000 g \times 120$ min in a Spinco SW-40 rotor. After centrifugation, 20–22 ten-drop fractions were collected and the radioactivity in each fraction counted. Up to 58% was recovered in a single peak in 1.33 M sucrose, with uniform distribution of radioactivity in the other fractions. In another experiment using ^{14}C labelled 5-HT, we also found a single peak of radioactivity in 1.3 M sucrose. Electron microscopy of the 20,000 g pellet used for preparation of the sucrose density gradient showed a population of subcellular particles including endoplasmic reticulum, α -granules, dense-cored

storage organelles, and some mitochondria. Maynert & Isaac (1968) obtained a comparable mixture of subcellular particles in experiments with sonified rabbit platelets.

Discussion.—We have now demonstrated that dopamine is taken up by cat platelets *in vivo* as well as *in vitro*. This supports our earlier work with human platelets, where a dopamine-like substance was found in cells isolated from blood of parkinsonian patients receiving approximately 4 g/day of L-dopa orally (Boullin & O'Brien, 1970).

The rapid uptake may be responsible for the initial decline in plasma dopamine, as a substantial proportion of circulating unmetabolized dopamine is present in the platelets at all times after infusion. Thus, platelet dopamine accumulation may play an important role in removing the amine from the circulation. If this is in fact the case, the physiological and clinical significance of platelet dopamine must be considered.

We have previously reported that, in contrast to 5-HT, platelets lose considerable amounts of accumulated dopamine *in vitro* (Boullin & O'Brien, 1970). In this regard, it is interesting that the losses of dopamine during the ultracentrifugation procedure are greater than the losses of 5-HT (Maynert & Isaac, 1968). Nevertheless, we have been able to demonstrate that a large proportion of the radioactivity associated with the 20,000 g pellets is recovered in the fraction which is known to contain platelet 5-HT storage granules (Maynert & Isaac, 1968). We conclude that platelets take up dopamine *in vivo* and that at least part of the amine can be recovered from a subcellular platelet fraction rich in granules; thus, dopamine may be stored in the same structures that contain 5-HT.

REFERENCES

- BOULLIN, D. J. & O'BRIEN, R. A. (1970). Accumulation of dopamine by blood platelets from normal subjects and parkinsonian patients under treatment with L-DOPA. *Br. J. Pharmac. Chemother.*, **30**, 779–788.
- MAYNERT, E. W. & ISAAC, L. (1968). Uptake and binding of serotonin by the platelet and its granules. In *Advances in Pharmacology* 6A, ed. Garattini, S. and Shore, P. A. pp. 113–122. New York: Academic Press.
- ROBINSON, D. S., LOVENBERG, W., KEISER, H. & SJOERDSMA, A. (1968). Effects of drugs on human blood platelet and plasma amine oxidase activity *in vitro* and *in vivo*. *Biochem. Pharmac.*, **17**, 109–119.

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