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## Inhibition of [<sup>3</sup>H]dopamine uptake by platelets by the dopamine-D<sub>2</sub> receptor agonist RU 24926

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**Abstract**—We have examined the effect of the dopamine-D<sub>2</sub> receptor agonist RU 24926 (*N*-*n*-propyl-di- $\beta$ (3-hydroxy-phenyl)-ethylamine HCl) on [<sup>3</sup>H]dopamine uptake by human platelets. RU 24926 reduced the uptake of [<sup>3</sup>H]dopamine by platelet-rich plasma and this effect was not reversed by the dopamine-D<sub>2</sub> receptor antagonist haloperidol, or the dopamine-D<sub>1</sub> receptor antagonist SCH 23390 (8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol). These data suggest that RU 24926 reduces [<sup>3</sup>H]dopamine uptake by platelets by competing for the dopamine uptake mechanism on the platelet and not by activation of the dopamine-D<sub>2</sub> receptor.

Dopamine uptake by human platelets has been shown to be by an energy and temperature process (Solomon et al 1970; Dean & Copolov 1989) which does not involve binding to the dopamine-D<sub>1</sub> or D<sub>2</sub> receptor (Dean & Copolov 1989). To determine if

activation of the D<sub>2</sub> receptor could modulate uptake by platelets we have examined the effect of the D<sub>2</sub> receptor agonist, RU 24926 (Euvard et al 1980), on the uptake of [<sup>3</sup>H]dopamine by human platelets.

### Materials and methods

The studies were approved by the human ethics committee of Royal Park Hospital, and the volunteers gave written, informed consent.

[<sup>3</sup>H]Dopamine uptake by platelets was measured as described previously (Dean & Copolov 1989). Blood was obtained from volunteers selected at random from 59 individuals. At the time of donation the volunteers had no current medical problems and had not taken drugs for at least three weeks. Platelet rich plasma (PRP) was taken as the supernatant after centrifuging the blood, which had been anti-coagulated with EDTA, at 100 *g* for 15 min.

Samples of PRP (250  $\mu$ L) were incubated with a range of concentrations of unlabelled dopamine or RU 24926 (0–10  $\mu$ M)

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for 10 min at 37°C. This procedure was performed in triplicate. [ $^3\text{H}$ ]Dopamine was then added (final concn 9 nM, final volume 300  $\mu\text{L}$ ) and the incubation continued for 60 min at 37°C. The uptake of [ $^3\text{H}$ ]dopamine was terminated by placing the assay tubes on ice and then centrifuging at 18 000 g for 5 min at 4°C. The supernatant was aspirated and 250  $\mu\text{L}$  distilled water added to the platelet pellet which was then frozen and thawed. The re-suspended platelet pellet was dispersed in 5 mL of Beckman Readiprotein and the radioactivity counted in a Packard 1500 LSC. The radioactivity in each tube was taken as a measure of total dopamine uptake by the platelets. Non-specific trapping of dopamine was taken as the radioactivity in the platelet pellets of PRP maintained at 4°C and centrifuged immediately after the addition of radiolabelled dopamine. Specific uptake of [ $^3\text{H}$ ]dopamine was taken as total uptake minus non-specific trapping and expressed as pmol/ $10^{11}$  platelets. To allow comparison of results between individuals specific uptake was expressed as a percentage of control, control being uptake in the presence of [ $^3\text{H}$ ]dopamine alone.

[ $^3\text{H}$ ]Dopamine uptake was also measured using the above method but in the presence of 10  $\mu\text{M}$  of RU 24926 (*N*-n-propyl-di- $\beta$ (3-hydroxy-phenyl)-ethylamine HCl) or dopamine with or without (10  $\mu\text{M}$ ) haloperidol or SCH 23390 (8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol).

Statistical analysis was carried out using Student's *t*-test.

### Results and discussion

[ $^3\text{H}$ ]Dopamine uptake by platelets was inhibited in a dose-dependent manner by both unlabelled dopamine and RU 24926 (Fig. 1) from a level of 305 pmol/ $10^{11}$  platelets to 120 and 114 pmol/ $10^{11}$  platelets, respectively. RU 24926 appeared equipotent in inhibiting the uptake of [ $^3\text{H}$ ]dopamine as unlabelled dopamine (ED $_{50}$  5.0 vs 5.3  $\mu\text{M}$ ). In addition, the ability of RU 24926 to significantly reduce the uptake of [ $^3\text{H}$ ]dopamine by platelets could not be reversed by haloperidol or SCH 23390 (Table 1)

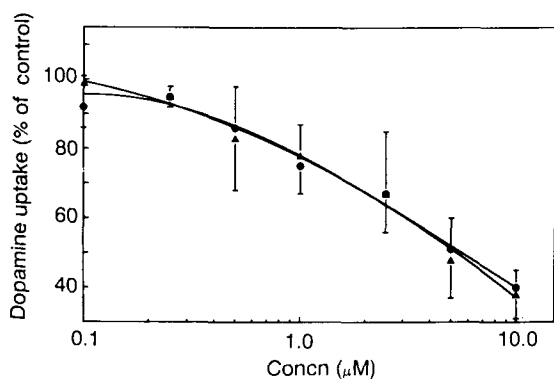


Fig. 1. The effect (mean  $\pm$  s.d.) of varying concentrations of the dopamine receptor agonists dopamine ( $\bullet$ — $\bullet$ ) and RU 24926 ( $\blacktriangle$ — $\blacktriangle$ ) on the uptake of [ $^3\text{H}$ ]dopamine by platelet-rich plasma from 5 individuals.

Table 1. The effect (mean  $\pm$  s.d.) of RU 24926 (10  $\mu\text{M}$ ) and dopamine (10  $\mu\text{M}$ ) on [ $^3\text{H}$ ]dopamine uptake by platelet-rich plasma from 5 individuals in the presence (10  $\mu\text{M}$ ) and absence of haloperidol and SCH 23390.

	[ $^3\text{H}$ ]Dopamine uptake by platelets (% control)	<i>P</i>
Control	100 $\pm$ 8	
RU 24926	40 $\pm$ 17	<0.001
RU 24926 + haloperidol	37 $\pm$ 13	<0.001
RU 24926 + SCH 23390	45 $\pm$ 8	<0.001
Dopamine	42 $\pm$ 6	<0.001
Dopamine + haloperidol	35 $\pm$ 5	<0.001
Dopamine + SCH 23390	34 $\pm$ 12	<0.001

suggesting their effects were not caused by activating either the D $_2$  or D $_1$  receptor. Thus, it would seem RU 24926, a reported D $_2$  receptor agonist (Euvard et al 1980), reduces [ $^3\text{H}$ ]dopamine uptake by platelets by competing with dopamine for the dopamine uptake mechanism of the platelet. As this dopamine mechanism of the platelet appears to have homologies with the dopamine transporter of striatal synaptic vesicles (Dean et al 1990), it would seem possible that RU 24926 could also interact with the dopamine transporter in the CNS.

RU 24926 has been reported to have non-D $_2$  receptor effects in the CNS (Martin & Bendesky 1984) as sulpiride, a D $_2$  receptor antagonist, was unable to reverse the ability of RU 24926 to increase mouse locomotor activity.

In conclusion it would appear that RU 24926 reduces [ $^3\text{H}$ ]dopamine uptake by the platelet by interacting with the dopamine uptake mechanism and therefore may provide a novel marker in studies designed to identify and purify the platelet dopamine transporter. Furthermore, RU 24926 could prove to be an inhibitor of the dopamine transport mechanism in the CNS.

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