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ptake of dopamine by rat hepatocytes *in vitro*

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BSTRACT The present results showed that ptake of dopamine (DA) by rat isolated hepocytes was mediated, in addition to simple ffusion, mainly by a transporter-involved ocess, with K_m of 66.8 μmol and V_{max} of .3 pmol·min^{-1}/10^5 cells. The process was H- and temperature-dependent and required activation energy of 4.12 kcal·mol^{-1} (Q_{10} = 25) in the range of 2.0—12.7 ℃ and 13.0 al·mol^{-1} (Q_{10} = 2.0) in the range of 12.7—9.0 ℃. Cysteine residue having free thiol roup was unrelated to the activity of the nsporter. Catecholamines, serotonin, and caine inhibited the DA transport, but ramine (TA) and tryptamine, as well as enztropine and imipramine (which are potent hibitors for hepatic TA transporter and euronal DA transporter), had no inhibitory ffect on the transport of DA in these cells. hese results indicated that DA was taken up to hepatocytes by a distinct carrier. NaF nd mastoparan influenced the transport activy in these cells further, suggesting that sigal transducing G-proteins may be involved in he regulation of DA transporter in rat hepatoytes.

EY WORDS liver; cultured cells; opamine; biological transport; wasp venoms

It is well established that the concentration of dopamine (DA) in brain is controlled the intercellular space mostly by reuptake echanisms. The neuronal DA transporters of human[1] and rat[2] have been cloned. In contrast, studies of DA transporters in extraneuronal tissues are rare except the uptake of DA in platelets which was altered in patients with Parkinson disease, schizophrenia, and Huntington's disease[3]. About 50 % of DA uptake into platelets was mediated through a serotonin carrier, while the other 50 % is possibly due to passive diffusion and low affinity uptake[4].

DA is used widely for treatment of shock and heart failure. Following intravenous injection, DA is mainly cleared from plasma by extraneuronal tissues, among which liver plays an important role. As a consequence of the entry of DA into liver cells, the amine is inactivated by monoamine oxidase of the outer membrane of mitochondria. Information concerning the mechanisms by which DA is transported through the hepatic plasma membrane is not available. Recently, we have characterised a transport system in rat hepatocytes for the uptake of tyramine (TA)[5]. Since TA is structurally very similar to DA, we proceeded to study the DA uptake by rat isolated hepatocytes.

MATERIALS AND METHODS

Wistar rats, ♂, weighing about 200 g, were starved for 20 h before killing. Hepatocytes were isolated according to the method of Seglen[6] with slight modification: both Ca^{2+}-free and collagenase perfusions were carried out at 34 ℃ for 6 min. Only the preparations in which > 90 % of the cells excluded trypan blue were used. The cells were kept at 4 ℃ and within 2 h.

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ures, transport assays were performed in RPMI 1640 medium without serum, containing HEPES 10 mmol $\cdot L^{-1}$ (pH 7.6), at 37 C for 30 s. In this case, the amounts of [^{3}H]DA within the cells after incubation did not exceed 5 % of the amount added to the medium. Cells (3×10^5) were preincubated at 37 C for 2 min and then incubated with [^{3}H]DA 30 nmol $\cdot L^{-1}$ (specific radioactivity 1.25 TBq $\cdot mol^{-1}$) in a final volume of 200 μl, in the absence or presence of inhibitors which were added simultaneously with the labelled TA, except when indicated in the legends. The uptake reaction was stopped by the addition of 400 μl ice-cold medium. Samples of 200 μl were immediately put in an Eppendorf microtube, on the top of 150 μl oil (a mixture of dibutyl phthalate and dioctyl phthalate of 1.20 g $\cdot ml^{-1}$ density) and centrifuged at 10 000 $\times g$ for 30 s. The cell pellet was cut and added to 0.5 ml boiling SDS solution (10 %) in a scintillation vial. After 5 min, 4 ml of a liquid-scintillation cocktail (Aqualuma Lumac, Landgraaf, The Netherlands) were added and counted in a Beckman β counter. When the effects of thiol group modifier was tested, experiments were carried out with perfusion buffer which was composed of NaCl 137, KCl 5.4, $Na_2HPO_4\cdot2H_2O$ 3.4, KH_2PO_4 3.5, $MgSO_4\cdot7H_2O$ 8.1, $NaHCO_3$ 24.4, and $CaCl_2\cdot2H_2O$ 2.0 mmol $\cdot L^{-1}$, pH: 7.4. Buffer X[7] used for the determination of mastoparan[7] effect on DA transport in hepatocytes contains NaCl 4.74, KCl 118, $CaCl_2$ 0.38, egtazic acid 1, $MgSO_4$ 1.19, KH_2PO_4 1.19, sodium pyruvate 3, HEPES/KOH 25 mmol $\cdot L^{-1}$, pH: 7.4[8]. Experiment concerning the effects of F^- on DA transport in hepatocytes was performed in HEPES buffer (20 mmol $\cdot L^{-1}$, pH: 7.4) containing NaCl 40 mmol $\cdot L^{-1}$ and different concentraions of sucrose to keep the osmolarity balance of the solution (300 mOsm $\cdot L^{-1}$). The extracellular water contamination was measured by adding [^{14}C]sucrose to a hepatocyte suspension before centrifugation and radioactivity determination in the cell pellet. Less than 0.3 % of the added radioactivity was present in the pellet. Thus, the contamination with extracellular water is always substantially lower ($n=3$, 2.02 ± 0.10 %) than the total radioactivity associated with the cell pellet in the [^{3}H]DA uptake experiments.

Since simple diffusion and transporter mediated processes coexist in the uptake of DA by hepatocytes, totall DA transport refers to both processes involved in transport, while DA transport refers to the transporter mediated uptake which is calculated after eliminating the simple diffusion part measured in the presence of cold DA 10 mmol $\cdot L^{-1}$.

RESULTS

Uptake kinetics of [^{3}H]DA [^{3}H]DA was incubated with isolated hepatocytes at 37 C. The accumulation of [^{3}H]DA in the cells was time-dependent (Fig 1). The cellular influx rates were determined as a function of DA concentration in the medium (Fig 2). The curve was biphasic and could be resolved into a linear and a hyperbolic curve with a K_m of 66.8 μmol $\cdot L^{-1}$ and V_{max} of 523 pmol $\cdot min^{-1}$/10^5 cells, suggesting that both simple diffusion and transporter-mediated mechanisms are involved in this uptake process.

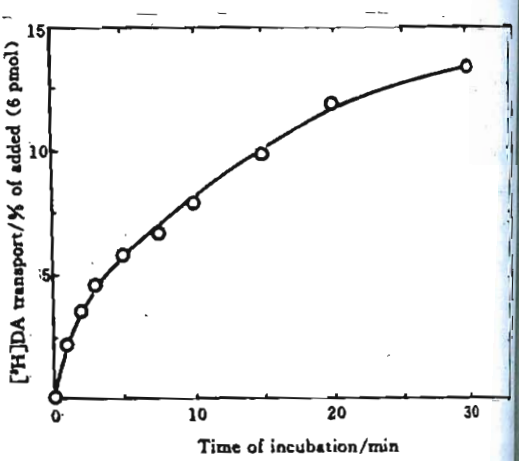

Fig 1. [^{3}H]DA uptake by isolated hepatocytes incubated with [^{3}H]DA (30 nmol $\cdot L^{-1}$) at 37 C in RPMI 1640 medium.

Effects of temperature and extracellular pH on [^{3}H]DA transport [^{3}H]DA transport in isolated hepatocytes was temperature-dependent, requiring an activation energy (E_a) of 4.12 kcal $\cdot mol^{-1}$ and Q_{10} of 1.25 (2.4 −12.7 C) as well as a E_a of 13.0 kcal $\cdot mol^{-1}$ and Q_{10} of 2.0 (12.7 − 39.0 C). The

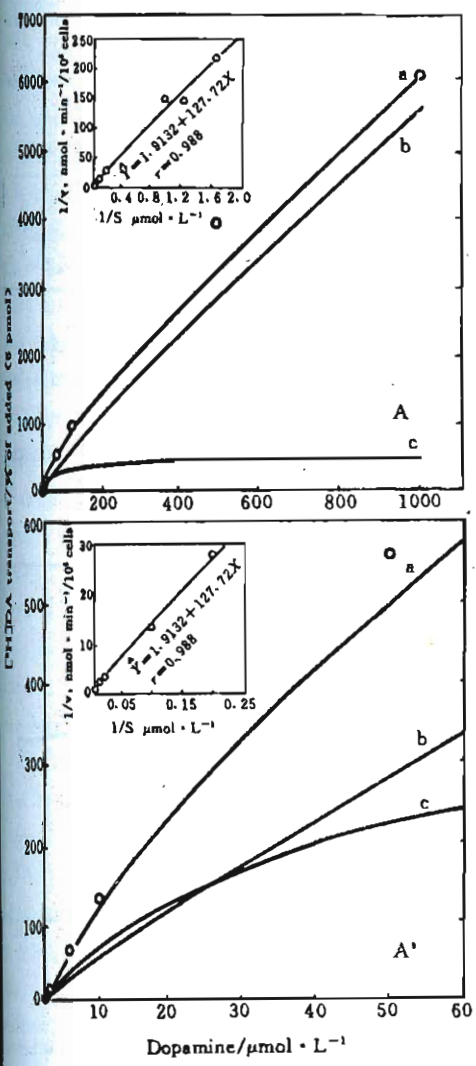

Fig 2. [³H]DA uptake by isolated hepatocytes. The curve (a) fitting the data (○) can be resolved into a saturable process (c) with a K_m of 66.8 μmol·L^{-1} and a V_{max} of 523 pmol·min^{-1}/10^5 cells as well as a linear uptake (b): v (pmol·min^{-1}/10^5 cells) = 5.61× [S]. A': an inset from A.

[³H]DA transport was also influenced by the pH of the medium. The [³H]DA transport activity at 37 ℃ quickly increased as the pH in the medium rose from 6.44 to 7.25, reaching a plateau after pH 7.49. The increase of [³H]DA transport at 4℃ was considerably slower with increasing pH (Fig 3).

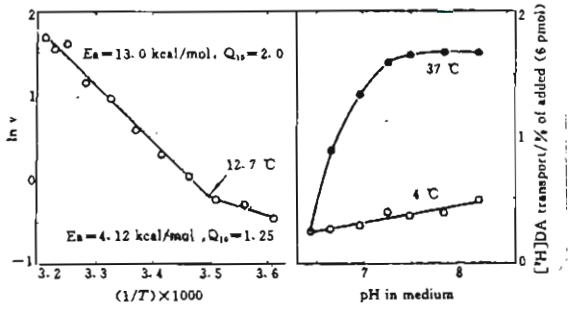

Fig 3. Effects of temperature and extracellular pH on [³H]DA uptake by hepatocytes.

Inhibition of [³H]DA transport by DA analogues and other substances Tested on [³H]DA transport in isolated hepatocytes were DA analogues and non-analogues including substances which potentially block TA tranpsorters in rat liver cells[5] and DA transporters in brain[9], such as benztropine, imipramine, and cocaine, as well as the substances which modify the thiol group of cysteine, such as *N*-ethylmaleimide (NEM), *p*-aminophenylmercuric acetate (pAPMA), ethylmercurithiosalicyclic acid (EMTSA), and iodoacetamide. We used thiol group modifiers because the activity of many transporters, such as hepatic tyramine transporters[5], depends on the cysteine residue having free thiol group within the polypeptides. The transport of [³H]DA in the hepatocytes was most potentially inhibited by cold DA, and it was also inhibited by serotonin, adrenaline, noradrenaline, and cocaine, but not by TA, tryptamine, benztropine, and imipramine (Fig 4).

On the other hand, all the thiol-group modifiers did not modify the DA transport activity in rat hepatocytes (Tab 1).

Effect of F^- and mastoparan on [³H]DA transport Prior to the transport activity assay, the cells were incubated with Buffer X and mastoparan at 4 ℃ for 90 min immediate-

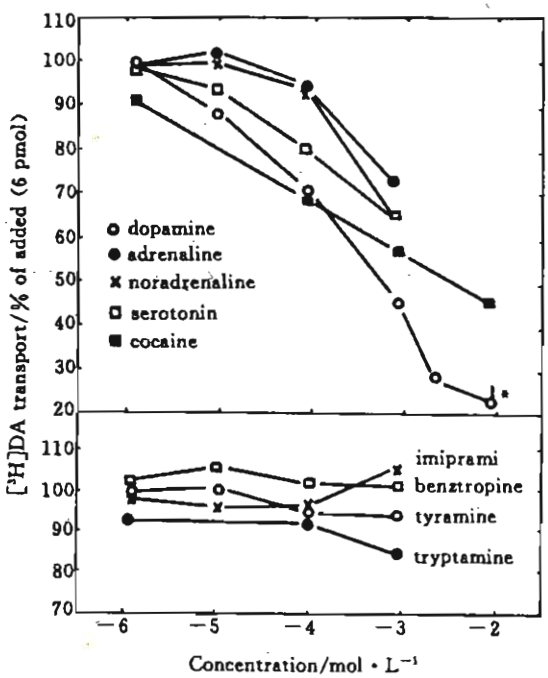

Fig 4. Effects of substances on uptake of [³H]DA by hepatocytes. $n=3$, $\bar{x}\pm s$.

Tab 1. Effects of amino acid modifiers on uptake of [³H]DA by 3×10^6 hepatocytes incubated in 2-ml perfusion buffer (pH: 7.6) at 25 ℃ for 30 min. Then the cells were washed twice with 5-ml perfusion buffer at 4 ℃ before transport assay $n=2$.

Modifier	Concentration/ mmol·L⁻¹	[³H]DA transport/ % of control (120 fmol)
NEM	1	95
pAPMA	1	99
EMTSA	1	125
Iodoacetamide	1	111

ly after the purification. As shown in Tab 2, mastoparan (80 μmol·L⁻¹) induced considerable inhibition of the DA transport, 42 ± 1.7% activity remained as compared with the control (Tab 2).

We also determined the influence on DA transport of F^-, which modulates the activity of adenylyl cyclase as the results of interaction of the anion with all G^- proteins[10]. F^-

Tab 2. Effects of mastoparan on [³H]DA uptake hepatocytes incubated in X buffer at 4 ℃ for 90 min. $n=3$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.01$ *vs* control.

Mastoparan/ μmol·L⁻¹	[³H]DA transport fmol	% of control
0 (control)	301±23	100±7.6
3.8	263±5[a]	87±1.7[a]
80	127±5[b]	42±1.7[b]

increased the DA transport activity. Th maximal activity (330 % of the control) w caused by NaF 20 mmol·L⁻¹. As the F^- co centration increased further, the transpo activity decreased (Fig 5).

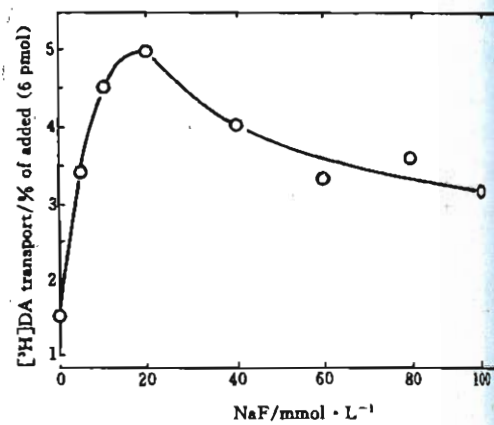

Fig 5. Effects of F^- on [³H]DA transport in hepatocytes.

DISCUSSION

The clearance of DA in general circulatio depends on the uptake processes of extra neuronal tissues, among which liver is an im portant organ. However, the mechanism b which DA is taken up by liver has never bee reported. The work presented here demon strated that the transport of DA in isolated r hepatocytes is mediated by a transporter-i volved process in addition to simple diffusion The physiological significance of this transpo

em is suggested by the following observa-
s. When the concentration of DA in
ium was $<25\ \mu mol \cdot L^{-1}$, the carrier-medi-
process was the main mechanism for the
ke of DA by hepatocytes (Fig 2). This
hithin the range of DA concentration in
ma of patients receiving intravenous injec-
of DA for the treatment of shock ($3.4-$
$\mu mol \cdot L^{-1}$)[11,12] or heart failure ($0.17-$
$4\ \mu mol \cdot L^{-1}$)[13]. Under the unsaturated
centration (30 $nmol \cdot L^{-1}$), 72 %—82 % of
]DA was captured through a transporter-
iated process and 18 %—28 % was taken
simple diffusion (Fig 4). Even in the
sence of high concentration of cold DA (10
$ol \cdot L^{-1}$), a small proportion of [^{3}H]DA
still represent nonspecific binding. The
proportion of [^{3}H]DA taken up by free
usion should be lower than the above-
itioned values.

For TA transport in isolated rat hepato-
s both simple diffusion and transporter-
iated processes are involved in the uptake
'A[5]. Are TA and DA taken up into rat
atocytes by the same carrier? Our results
gest 2 separate carriers are involved. This
lusion is supported by the following
ence:

1) DA potentially inhibits [^{3}H]TA trans-
in hepatocytes. In contrast, TA has no
bitory effect on the [^{3}H]DA transport in
e cells. Apparently, although DA has a
affinity for the TA transporter in these
. Apparently, although DA has a high
ity for the TA transporter, this trans-
er does not accept DA.

2) The activity of TA transporter expo-
ially increases with the increase of extra-
lar pH (6.3—7.9); The activity of DA
sporter exhibits a linear increase when the
um pH is below 7.25, and reaches a
au after pH 7.49.

3) The requirement of activation energy for the TA transporter appears to be homogenous from 2 to 38 ℃ ($E_a = 12.9$ $kcal \cdot mol^{-1}$ and Q_{10} and $Q_{10} = 2.0$); while that for the DA transporter exhibits biphasic behavior, ie, $E_a = 4.12$ $kcal \cdot mol^{-1}$ and $Q_{10} = 1.25$ (2.0—12.7 ℃) and $E_a = 13.0$ $kcal \cdot mol^{-1}$ and $Q_{10} = 2.0$ (12.7—39.0 ℃).

4) Benztropine, imipramine, and tryptamine are very potent inhibitors for TA transport but not DA transport indicating the structural difference between the 2 carriers.

5) Cysteine residue having free thiol group is necessary for the normal function of TA transporter in rat hepatocytes. Thiol group modifiers do not block the transport of DA, suggesting that this type of amino acid residue is not essential for the activity of the DA transporter.

More than 9 signal-transducing G-proteins have been indentified[14], among which G_s, and G_i (the stimulatory and the inhibitory regulatory components of adenylyl cyclase) are particularly important. Our results show that F^- 10—20 $mmol \cdot L^{-1}$ stimulated [^{3}H]DA transport in hepatocytes (Fig 5). This concentration is nearly identical to that (around 10 $mmol \cdot L^{-1}$) which stimulates glucose transporter through a G-protein modulated mechanism in rat epididymal fat cells[14,15]. As the concentration rises further, the transport activity decreases. We suppose that F^- at lower concentrations mainly activates G_s, causing the transport activity to increase. When the concentration of F^- increases, G_i is also activated, inducing the transport activity to decrease. The result is in agreement with the finding that mastoparan inhibited DA transport in hepatocytes (Tab 2). Mastoparan is more active in stimulating G_i (8—10 times) than G_s (2 times)[17]. Thus it is possible that only the inhibitory effect caused by this

peptide toxin can be seen when both G_s and G_i are involved in regulating the activity of the transport.

In conclusion, a transporter-mediated process is the main mechanism for the uptake of DA in hepatocytes. The carrier responsible for the transport of DA is different from that for the uptake of TA in these cells. Signal transducing G-proteins are probably involved in regulating the DA transporter in rat hepatocytes.

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