J. Pharm. Pharmacol. 1998, 50: 1027–1033
Received January 28, 1998
Accepted April 30, 1998

Efflux Transport of Tolbutamide Across the Blood–brain Barrier

HITOMI TAKANAGA, HIDEYASU MURAKAMI, NORIKO KOYABU, HIROTAMI MATSUO, *MIKIHIKO NAITO, *TAKASHI TSURUO AND YASUFUMI SAWADA

*Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka-shi 812-8582, and *Institute of Molecular Biosciences, University of Tokyo, Tokyo 133-0031, Japan*

Abstract

In an attempt to determine the reason for the low brain distribution of tolbutamide, we have demonstrated the transport of tolbutamide from the brain to the blood via a non-P-glycoprotein efflux transport system which is inhibited by sulphonamides. We evaluated the directional transport of tolbutamide across the blood–brain barrier by means of an in-vivo brain-tissue distribution study and experiments on in-vitro transcellular transport and uptake in cultured mouse-brain capillary endothelial cells (MBEC4).

The brain-to-unbound-plasma concentration ratio of [^{14}C]tolbutamide increased in the presence of high concentrations of unlabelled tolbutamide or sulphonamide at steady-state in-vivo. The brain-to-blood concentration ratios of [^{14}C]tolbutamide were very low compared with that of [^{3}H]propranolol obtained by in-vivo integration plot analysis. From the in-vitro transcellular transport study using a monolayer of MBEC4 cells, we found that the abluminal-to-luminal flux of [^{14}C]tolbutamide was higher than the reverse flux. Both luminal-to-abluminal and abluminal-to-luminal transport of tolbutamide were saturable. The maximum transport rate (J_{max}), the half-saturation concentration (K_t), and the first-order rate constant (k_d) were $65{\cdot}9 \pm 29$ pmol min^{-1} (mg protein)$^{-1}$, $7{\cdot}54 \pm 4{\cdot}4$ μM, and $4{\cdot}89 \pm 0{\cdot}34$ μL min^{-1} (mg protein)$^{-1}$, respectively, for luminal-to-abluminal transport, and 128 ± 66 pmol min^{-1} (mg protein)$^{-1}$, $5{\cdot}59 \pm 4{\cdot}2$ μM, and $4{\cdot}43 \pm 0{\cdot}86$ μL min^{-1} (mg protein)$^{-1}$, respectively, for abluminal-to-luminal transport. At therapeutic plasma concentrations of tolbutamide (1–16·9 μM), the efflux rate would be faster than the influx rate. The estimated net efflux was consistent with the very low in-vivo brain distribution of tolbutamide. The efflux process observed in MBEC4 cells was inhibited by sulphonamides such as sulphaphenazole, sulphamethoxazole and sulpha-dimethoxine whereas the steady-state uptake of [^{14}C]tolbutamide was not affected by either cyclosporin or verapamil, specific inhibitors of P-glycoprotein.

These findings suggest that tolbutamide is partly transported from the brain via the non-P-glycoprotein-efflux transport system, which is inhibited by sulphonamides.

Sulphonylureas are frequently used in the treatment of diabetes. Incidents of severe hypoglycaemia have been reported as a result of co-administration of tolbutamide and sulphaphenazole. Two possible mechanisms have been postulated for the drug–drug interaction—inhibition of the oxidation of tolbutamide by P450 (CYP2C9) by sulphaphenazole and displacement of binding of tolbutamide to serum protein by sulphaphenazole, with the result that sulphaphenazole prolongs the time of effective concentration of unbound tolbutamide in the blood (Rowland & Martin 1973; Shibasaki et al 1977; Sugita et al 1981). We have previously developed a physiological pharmacokinetic model to study the effect of sulphonamide on the plasma elimination and tissue distribution of [^{14}C]tolbutamide in the rat (Sugita et al 1982). The tissue-to-plasma unbound concentration ratio ($K_{p,f}$) of tolbutamide in a variety of organs or tissues was not significantly altered by the presence of sulphonamides such as sulphaphenazole, sulphamethoxazole and sulphadimethoxine whereas the $K_{p,f}$ value of tolbutamide in the brain was very low (0·1–0·2) compared with that for other organs or tissues such as heart, liver, kidney, spleen, pancreas, gastro-

Correspondence: Y. Sawada, Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka-shi 812-8582, Japan.

intestinal tract, adipose tissue, muscle and skin, and increased in the presence of a high concentration of tolbutamide or of sulphonamides (Sugita et al 1982). These observations might be explained by the brain-specific contribution of a transport mechanism for tolbutamide from the brain to blood.

The distribution of drugs to the brain is restricted by the blood–brain barrier, which is formed by brain capillary endothelial cells connected by a tight junction. Efflux transport systems such as that of P-glycoprotein at the blood–brain barrier, which transports selective molecules from the brain to blood circulation, cannot be disregarded (Tsuji et al 1992). The presence of such an efflux pump in the endothelial cells of the blood–brain barrier for preventing xenobiotics from entering the central nervous system raises important therapeutic and toxicological considerations. To explain the mechanism of the low brain distribution of tolbutamide it is important to study the transport of tolbutamide at the blood–brain barrier and to determine whether tolbutamide is transported from the brain via a specialized mechanism, such as P-glycoprotein. In this study we have evaluated the directional transport of tolbutamide across the blood–brain barrier, by means of on an in-vivo brain-tissue distribution study and experiments on transcellular transport, and measured in-vitro tolbutamide uptake by use of cultured mouse-brain capillary endothelial cells (MBEC4).

Materials and Methods

Materials

[ring-U-^{14}C]Tolbutamide (2·18 GBq mmol^{-1}), DL-[4-^{3}H]propranolol hydrochloride (1·04 TGBq mmol^{-1}) and [G-^{3}H]vincristine (226 GBq mmol^{-1}) were purchased from Amersham (Tokyo, Japan). 4-[*N*-Methyl-^{14}C]iodoantipyrine (2·5 GBq mmol^{-1}) and [^{14}C(U)]sucrose (0·17 GBq mmol^{-1}) were from DuPont–New England Nuclear (Boston, MA). Tolbutamide and verapamil were purchased from Nacalai Tesque (Kyoto, Japan). Sulphaphenazole, sulphamethoxazole and sulphadimethoxine were purchased from Sigma (St Louis, MO). Cyclosporin was a kind gift from Sandoz AG (Basel, Switzerland). All other chemicals were of reagent grade and commercially available.

Animal experiments

Experiments were performed on adult male Wistar rats, 250–300 g. Under slight ether and pentobarbital (1·25 mL kg^{-1}) anaesthesia the femoral vein and artery were cannulated with SP-10 and SP-31 polyethylene tubing, respectively. After administration of [^{14}C]tolbutamide (13·5 μg kg^{-1}) or [^{3}H]propranolol (0·0106 μg kg^{-1}) blood samples were collected from the artery in heparinized polyethylene centrifuge tubes. At a designated time the rats were killed by decapitation and their brains were rapidly removed. Cerebral blood flow was measured by the method of Sakurada et al (1978) by measuring the blood and brain concentrations of [^{14}C]iodoantipyrine after intravenous administration.

Cell culture

MBEC4 cells were cultivated as described elsewhere (Tatsuta et al 1992). Briefly, the cells were routinely grown in Dulbecco's modified eagle medium supplemented with 10% foetal bovine serum, penicillin (100 units mL^{-1}) and streptomycin (100 μg mL^{-1}) at 37°C in 5% CO_2–95% air.

For the transport study, MBEC4 cells were seed-ed at a density of 4×10^4 cells mL^{-1} on a polycarbonate membrane, Transwell clusters (1 cm^2/well), with a 3·0-μm pore size (Costar, Bedford, MA). The cells were grown for 3 days before the transport experiment. For the uptake study, MBEC4 cells were seeded at the same density on multidishes (Nunc, Denmark).

Transcellular transport and uptake experiments

Experiments on transport across MBEC4 cultured on Transwell or uptake by the cells on multidishes were performed in transport buffer (141 mM NaCl, 4 mM KCl, 2·8 mM $CaCl_2$, 1 mM $MgSO_4$, 10 mM D-glucose, 10 mM HEPES, pH 7·4) at 37°C. For quantitation of the drugs taken up by the cells the cells were solubilized with NaOH (5 M, 200 μL).

The amount of test compound transported or taken up by the cells was estimated from the radioactivity in the sample and expressed as the cell-to-medium ratio (μL (mg protein)$^{-1}$) by dividing the transported amount by the initial concentration in the donor compartment and correcting for the amount of cellular protein. Cellular protein was measured by the method of Lowry et al (1951) using bovine serum albumin as the standard.

Data analysis

Influx clearance (K_1) of the substrates into rat brain was estimated by means of an integration plot based on the relationship between the ratios of the concentrations in brain and blood (C_{brain}/C_{blood}) and AUC/C_{blood} according to the equation:

$$C_{brain}/C_{blood} = K_1(AUC/C_{blood}) \qquad (1)$$

where C_{brain} and C_{blood} are the concentrations in the brain and blood, respectively, and AUC is the area under the plot of blood concentration against time.

Cerebral blood flow (F/W) in the rat brain under anaesthesia was estimated by use of the equation (Sakurada et al 1978; Sawada et al 1987):

$$C_{brain}(T) = \lambda k \int C_{blood} e^{-k(T-t)} \quad (2)$$

where

$$k = (F/W) \times (m/\lambda) \quad (3)$$

F/W is the cerebral blood flow $(g\,brain)^{-1}$, m is a constant between 0 and 1 that represents the extent to which diffusional equilibrium between blood and brain is achieved by the marker material during its passage from the arterial to the venous end of the capillary, and λ is the tissue-to-blood concentration ratio for iodoantipyrine at the steady state. In this study, we took m as 1 and λ as 0·8 for iodoantipyrine in the normal brain. The k value in equation 2 was obtained by use of the non-linear least-squares regression analysis program, MULTI (Yamaoka et al 1981).

The transcellular transport rate was evaluated by linear regression analysis from the slopes of the initial linear portions of plots of amount transported against time. To estimate the kinetic parameters for saturable transport across MBEC4 cells, the transport rate (J) was fitted, by use of MULTI, to equation 4 that consisted of both saturable and non-saturable linear terms (Yamaoka et al 1981):

$$J = [J_{max}S/(K_t + S)] + k_dS \quad (4)$$

where J_{max} and K_t are the maximum transport rate and the half-saturation concentration, respectively, for the carrier-mediated process, S is the concentration of the substrate and k_d is the first-order rate constant. All data are expressed as means ± s.d. and statistical analysis was performed by use of Student's two-tailed *t*-test. A difference between means was considered to be significant when P values were $< 0{\cdot}05$.

Results

In-vivo study

Relationship between the tissue-to-plasma unbound concentration ratio for tolbutamide ($K_{p,f}$) and tissue concentration (C_{tissue}) in the rat. To investigate the possible presence of a saturable efflux transport system at the blood–brain barrier, we re-analysed our previous results (Sugita et al 1982). Figure 1A shows the relationship between the $K_{p,f}$ of tolbutamide and the brain concentration (C_{brain}) – $K_{p,f}$ increased with increasing C_{brain} and also increased in the presence of sulphaphenazole ($500\,\mu g\,mL^{-1}$) in plasma. A similar increase was obtained in the presence of sulphamethoxazole or sulphadimethoxine ($500\,\mu g\,mL^{-1}$). $K_{p,f}$ in muscle was, however, constant and was not affected by the sulphonamides (Figure 1B). These results indicate the presence of a brain-specific saturable efflux mechanism for tolbutamide which is inhibited by sulphonamides.

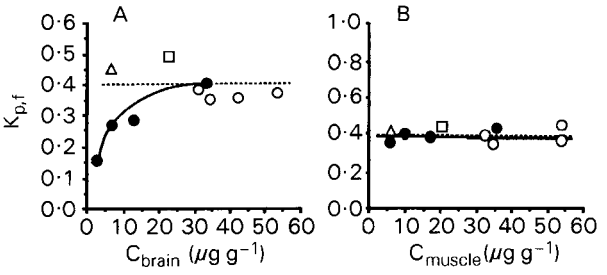

Figure 1. Tissue-to-plasma unbound concentration ratio ($K_{p,f}$) of tolbutamide in the rat. The $K_{p,f}$ of [^{14}C]tolbutamide in brain (A) and muscle (B) were measured in the absence (●) and presence of $500\,\mu g\,mL^{-1}$ (plasma concentration) sulphaphenazole (○), sulphamethoxazole (△) or sulphadimethoxine (□).

Integration plot of [^{14}C]tolbutamide uptake by rat brain. We investigated the uptake–time profiles of [^{14}C]tolbutamide by rat brain using an in-vivo integration plot. The brain-to-plasma concentration ratio for tolbutamide (K_p) rapidly reached approximately 0·02 as a quasi-equilibrium condition (Figure 2) whereas that for [^{3}H]propranolol increased linearly with time. The influx clearance (K_I) of [^{3}H]propranolol evaluated according to equation 1, $0{\cdot}42\,mL\,min^{-1}\,(g\,brain)^{-1}$, was comparable with the cerebral blood flow,

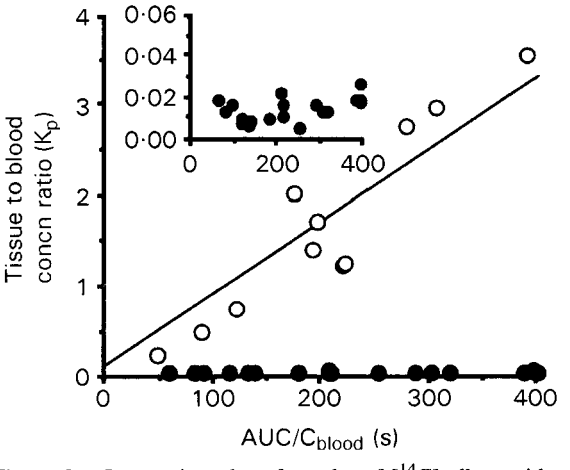

Figure 2. Integration plot of uptake of [^{14}C]tolbutamide and [^{3}H]propranolol by rat brain. After intravenous injection of [^{14}C]tolbutamide (●, $13{\cdot}5\,\mu g\,kg^{-1}$) or [^{3}H]propranolol (○, $10{\cdot}6\,ng\,kg^{-1}$), the brain-tissue-to-blood concentration ratio (K_p)–time profiles were obtained and the relationship between the K_p value and AUC/C_{blood} was plotted. The inset shows an expanded scale of K_p values from 0 to 0·06.

0·39 mL min^{-1} (g brain)$^{-1}$, calculated by use of equations 2 and 3.

In-vitro study with MBEC4 cells

Time-courses of transcellular transport of [^{14}C]tolbutamide. The time-course of [^{14}C]tolbutamide transport across the MBEC4 cell monolayers is shown in Figure 3. It is clearly apparent that the abluminal-to-luminal flux was higher than the reverse flux. The permeability coefficients for luminal-to-abluminal and abluminal-to-luminal were 14·8 ± 0·55 and 21·6 ± 1·6 μL min^{-1} (mg protein)$^{-1}$, respectively. These values were significantly higher than those for [^{14}C]sucrose (3·00 ± 0·33 and 3·05 ± 0·83 μL min^{-1} (mg protein)$^{-1}$, respectively, for luminal-to-abluminal and abluminal-to-luminal transport) which represent the paracellular permeability. Accordingly, most of the transport of [^{14}C]tolbutamide was ascribed to transcellular transport.

Concentration-dependence of tolbutamide transport across MBEC4 cells. Figure 4 depicts the relationship between the rate of transport and the concentration of tolbutamide (3–500 μM) after correction for paracellular permeability. Both directional permeabilities of tolbutamide consisted of two components, a saturable process at lower concentrations and an apparently non-saturable process at higher concentrations. The kinetic parameters for luminal-to-abluminal transport were J_{max} = 65·9 ± 28·9 pmol min^{-1} (mg protein)$^{-1}$ and K_t = 7·54 ± 4·36 μM for the saturable process and

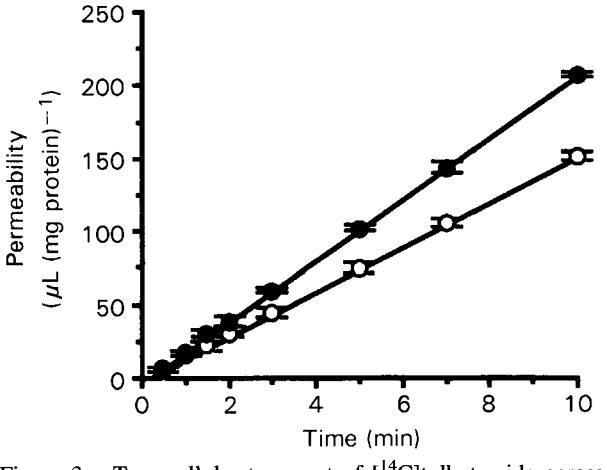

Figure 3. Transcellular transport of [^{14}C]tolbutamide across MBEC4 cells. The permeability to [^{14}C]tolbutamide (3 μM) was measured at 37°C. After applying [^{14}C]tolbutamide to the luminal (○; luminal-to-abluminal transport) or the abluminal (●; abluminal-to-luminal transport) sides of the cells, a sample was withdrawn from the abluminal side or the luminal side, respectively. Each point represents the mean ± s.e.m. of results from experiments in triplicate.

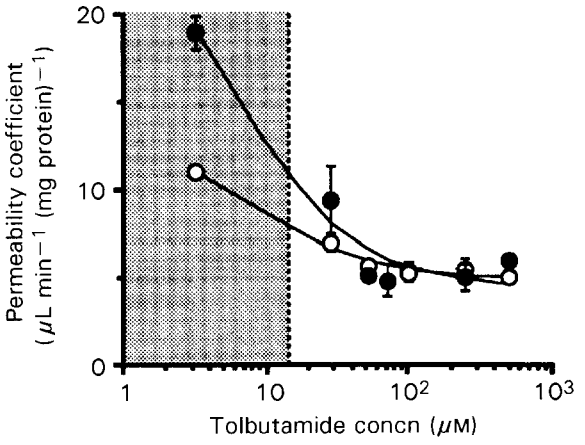

Figure 4. Concentration-dependence of transcellular transport of tolbutamide across MBEC4 cells. The incubation conditions were identical with those used for Figure 3. The rate of permeation of tolbutamide was measured from the slope of the time-course of transport in the luminal-to-abluminal (○) or abluminal-to-luminal direction (●). The solid lines represent the saturable component calculated from the kinetic parameters obtained. The shaded area indicates the range of therapeutic unbound plasma concentrations. Each point represents the mean ± s.e.m. of results from 3 or 4 experiments.

k_d = 4·89 ± 0·336 μL min^{-1} (mg protein)$^{-1}$ for the non-saturable process. Those for abluminal-to-luminal transport were J_{max} = 128 ± 65·8 pmol min^{-1} (mg protein)$^{-1}$, K_t = 5·59 ± 4·21 μM and k_d = 4·43 ± 0·863 μL min^{-1} (mg protein)$^{-1}$.

Inhibitory effect of sulphonamides on abluminal-to-luminal transport of [^{14}C]tolbutamide across MBEC4 cells. As shown in Figure 1, in-vivo $K_{p,f}$ values increased in the presence of sulphonamides (500 μg mL^{-1}) in plasma. To verify this observation the effect of sulphonamides on the ablumial-to-luminal transport of [^{14}C]tolbutamide was examined with MBEC4 cells (Figure 5). In the presence of sulphaphenazole or sulphamethoxazole the rate of transport of [^{14}C]tolbutamide was significantly inhibited to 60 or 70% of the control value. Sulphadimethoxine tended to inhibit tolbutamide flux, but the effect was not statistically significant. In the presence of 500 μg mL^{-1} sulphadimethoxine the rate of transport of [^{14}C]sucrose was 2·5-fold higher than in the control, presumably as a result of the toxic effect on the cells.

Inhibitory effect of verapamil, cyclosporin and p-aminohippuric acid on uptake of [^{14}C]tolbutamide by MBEC4 cells. The contribution of P-glycoprotein on tolbutamide efflux was examined by measuring the effects of verapamil and cyclosporin, specific P-glycoprotein inhibitors, on the steady-state uptake of [^{14}C]tolbutamide by MBEC4 cells. The cell/medium ratio for uptake of [^{3}H]vincris-

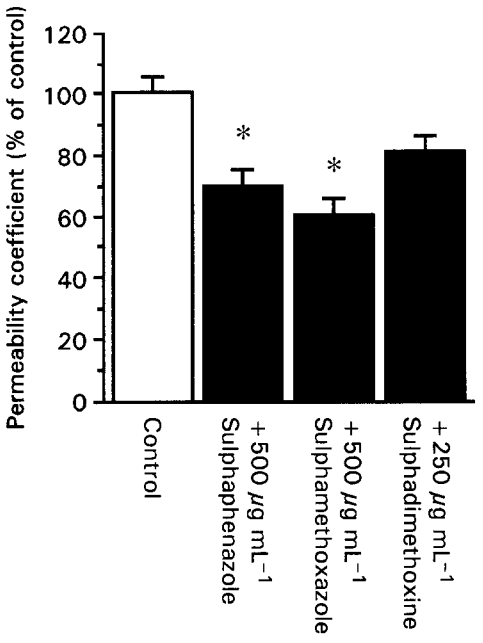

Figure 5. Effect of sulphonamides on transcellular transport of [^{14}C]tolbutamide across MBEC4 cells. The permeability coefficient of the control was $20{\cdot}0 \pm 0{\cdot}93\ \mu L\ min^{-1}$ $(mg\ protein)^{-1}$. Each point represents the mean ± s.e.m. of results from 3 or 4 experiments. $^*P < 0{\cdot}05$, significantly different from the control result (Student's *t*-test).

tine, a substrate of P-glycoprotein, at 1 h was $108 \pm 2{\cdot}0\ \mu L$ $(mg\ protein)^{-1}$, and it was significantly increased in the presence of 10 μM verapamil ($289 \pm 4{\cdot}4\ \mu L$ $(mg\ protein)^{-1}$) or 10 μM cyclosporin ($343 \pm 3{\cdot}5\ \mu L$ $(mg\ protein)^{-1}$). Accordingly, it was verified that the P-glycoprotein-mediated efflux is functioning in MBEC4 cells. The cell/medium ratio for [^{14}C]tolbutamide uptake at 1 h was $13{\cdot}4 \pm 0{\cdot}37\ \mu L$ $(mg\ protein)^{-1}$ and was not affected by 10 μM verapamil ($13{\cdot}4 \pm 0{\cdot}39\ \mu L$ $(mg\ protein)^{-1}$) or 10 μM cyclosporin ($12{\cdot}2 \pm 0{\cdot}55\ \mu L$ $(mg\ protein)^{-1}$). The effect of the anionic compound *p*-aminohippuric acid on [^{14}C]tolbutamide accumulation was also measured. The cell/medium ratios for [^{14}C]tolbutamide uptake at 1 h were $10{\cdot}9 \pm 0{\cdot}49$ and $10{\cdot}9 \pm 0{\cdot}45\ \mu L$ $(mg\ protein)^{-1}$, respectively, in the absence and presence of 1 mM *p*-aminohippuric acid. Accordingly, tolbutamide seems to be transported by a mechanism other than the P-glycoprotein or the *p*-aminohippuric acid-sensitive anion efflux system.

Discussion

This study has used in-vivo tissue distribution and in-vitro blood–brain barrier transport experiments to investigate the mechanism of the non-linear brain distribution of tolbutamide and the effect of sulphonamide on this mechanism. Our previous observation of the effect of sulphonamides on the plasma elimination and tissue distribution of [^{14}C]tolbutamide in the rat (Sugita et al 1982) has been re-analysed. The brain distribution, expressed as $K_{p,f}$, after intravenous administration of [^{14}C]tolbutamide was less than unity (0·1–0·2) and increased non-linearly with increased total brain concentration and in the presence of sulphonamides at the steady state in-vivo (Figure 1). The low $K_{p,f}$ of tolbutamide in the brain at steady state might be attributed to several mechanisms, the pH difference between the blood and the brain tissue, the existence of intracellular space impermeable to tolbutamide, or heterogeneous tolbutamide distribution in the brain (e.g. nerve cell). Furthermore, although C_{brain} in the presence of sulphaphenazole was higher than in the presence of sulphamethoxazole or sulphadimethoxine, $K_{p,f}$ in the presence of sulphaphenazole was lower than in the presence of sulphamethoxazole or sulphadimethoxine. This phenomenon cannot be explained merely by an efflux mechanism across the blood–brain barrier, and one possibility is an uptake mechanism inhibited by sulphaphenazole. The $K_{p,f}$ in other tissues was also less than unity, but did not show saturable concentration-dependence in this concentration range ($C_{p,f}$ 10–150 $\mu g\ mL^{-1}$) or an increase in $K_{p,f}$ in the presence of sulphonamides. The K_p value obtained from the integration plot for tolbutamide was low and rapidly reached steady state, 0·02 (Figure 2). Such low permeability to tolbutamide is unusual considering its lipophilicity (logP 2·34 at pH 7·4; Hansch & Leo 1979). Because these observations could be explained by the existence of an extensive efflux transport system to remove tolbutamide from the brain, we investigated the blood–brain barrier transport of tolbutamide in detail.

It has been demonstrated that cultured brain capillary endothelial cells, MBEC4, form a polarized monolayer on polycarbonate porous filters, with their abluminal membrane facing the filter and the luminal membrane to the culture medium (Tatsuta et al 1992). Such a cell monolayer is advantageous for study of directional transcellular transport in which the molecule can have free and selective access to either the luminal or abluminal membrane of the cell monolayers (Shirai et al 1994). When tolbutamide flux across the MBEC4 cell monolayer was measured, the permeability coefficient ($21{\cdot}6 \pm 1{\cdot}6\ \mu L\ min^{-1}$ $(mg\ protein)^{-1}$) from the abluminal-to-luminal side was significantly greater than that from the luminal -to-abluminal side ($14{\cdot}8 \pm 0{\cdot}55\ \mu L\ min^{-1}$ $(mg\ protein)^{-1}$) (Figure 3), revealing unidirectional transport from the brain to the blood. As shown in

Figure 4, we found that both directional fluxes include at least one saturable mechanism. The permeabilities estimated from the J_{max}/K_t values for luminal-to-abluminal and abluminal-to-luminal transport of tolbutamide were 8·7 and 23 μL min^{-1} (mg protein)$^{-1}$, respectively, values which suggest that a saturable component mainly contributes to the net efflux transport of tolbutamide across the blood–brain barrier. Within the range of the therapeutic unbound plasma concentration of tolbutamide (1–16·9 μM), the flux of tolbutamide from the abluminal-to-luminal side is expected to dominate the reverse flux (Figure 4). Permeability to [^{14}C]tolbutamide was inhibited by sulphaphenazole, sulphamethoxazole and sulphadimethoxine (Figure 5), results consistent with an in-vivo increase in the brain distribution of [^{14}C]tolbutamide in the presence of sulphonamides (Figure 1).

It was recently reported that P-glycoprotein at the blood–brain barrier is connected with low brain in-vivo distribution of anticancer agents such as vincristine, cyclosporin, doxorubicin and digoxin (Schinkel et al 1994, 1995; Ohnishi et al 1995). It acts as an ATP-dependent efflux pump to export anticancer agents and reduces their brain concentrations to a sublethal level. Because it is important to clarify whether tolbutamide is a substrate for P-glycoprotein, we examined the effect of verapamil and cyclosporin, specific inhibitors of P-glycoprotein, on tolbutamide uptake by MBEC4 cells. Although the accumulation of [^{14}C]tolbutamide at steady state by MBEC4 cells was not influenced by verapamil or cyclosporin, that of [^{3}H]vincristine was significantly increased. Various compounds with different structures are transported by the P-glycoprotein-mediated efflux system. Although lipophilicity and cationic properties have been assumed to be important if compounds are to be substrates for P-glycoprotein, the essential chemical structure required for recognition of the substrate is still unclear. Because tolbutamide is a lipophilic anionic compound, the lack of a contribution by P-glycoprotein might be reasonable, and another anionic-compound-specific efflux mechanism for tolbutamide at the blood–brain barrier might be a possibilty. Several investigators have suggested that there are functional differences in organic anion transport among tissues (Prichard & Miller 1993; Takano et al 1994). Although it has been reported that *p*-aminohippuric acid is removed by probenecid-sensitive organic-anion transport across the blood–brain barrier (Kakee et al 1996; Deguchi et al 1997), we found that [^{14}C]tolbutamide uptake was not inhibited by *p*-aminohippuric acid in the current study. If tolbutamide is transported through the probenecid-sensitive organic-anion-transport mechanism in the brain, the concentration of *p*-aminohippuric acid used in the current study is expected to be high enough to inhibit tolbutamide uptake, which implies that tolbutamide is removed by a mechanism other than the P-glycoprotein or *p*-aminohippuric acid transporters, and details of the mechanism of transport of tolbutamide must be investigated. Recently, a sulphonylurea receptor was identified (Aguilar-Bryan et al 1995). The affinity of tolbutamide binding to the receptor was of the order of micromolar, i.e. comparable with the K_t value obtained in this study. It might be of interest to investigate similarities between the biochemical and pharmacological characteristics of the sulphonylurea receptor and the transporter of sulphonylurea at the blood–brain barrier.

In conclusion, we have demonstrated that tolbutamide is removed from the brain to the blood by a non-P-glycoprotein-efflux transport system which is inhibited by sulphonamides. This specific transport system probably facilitates efflux of tolbutamide from the brain, resulting in the limited brain distribution of tolbutamide and causing a drug–drug interaction between tolbutamide and sulphonamide in respect of brain accumulation.

Acknowledgements

This work was supported in part by grants-in-aid for scientific research from the Ministry of Education, Science and Culture, Japan, and from the Takeda Science Foundation, and the Mochida Memorial Foundation for Medical and Pharmaceutical Research.

References

Aguilar-Bryan, L., Nichols, C. G., Wechsler, S. W., Clement, J. P. 4th, Boyd, A. 3rd, Gonzalez, G., Herrera-Sosa, H., Nguy, K., Bryan, J., Nelson, D. A. (1995) Cloning of the beta cell high-affinity sulphonylurea receptor: a regulator of insulin secretion. Science 268: 423–426

Deguchi, Y., Nozawa, K., Yamada, S., Yokoyama, Y., Kimura, R. (1997) Quantitative evaluation of brain distribution and blood-brain barrier efflux transport of probenecid in rats by microdialysis: possible involvement of the monocarboxylic acid transport system. J. Pharmacol. Exp. Ther. 280: 551–560

Hansch, C., Leo, A. (1979) Substituent Constants for Correlation Analysis in Chemistry and Biology. Appendix II Partition Coefficients. John Wiley and Sons, New York

Kakee, A., Terasaki, T., Sugiyama, Y. (1996) Brain efflux index as a novel method of analysing efflux transport at the blood-brain barrier. J. Pharmacol. Exp. Ther. 277: 1550–1559

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265–275

Ohnishi, T., Tamai, I., Sakanaka, K., Sakata, A., Yamashima, T., Yamashita, J., Tsuji, A. (1995) In vivo and in vitro evidence for ATP-dependency of P-glycoprotein-mediated efflux of doxorubicin at the blood-brain barrier. Biochem. Pharmacol. 49: 1541–1544

Prichard, J. B., Miller, D. S. (1993) Mechanisms mediating renal secretion of organic anions and cations. Physiol. Rev. 73: 765–796

Rowland, M., Martin, S. B. (1973) Kinetics of drug–drug interactions. J. Pharmacokinet. Biopharm. 1: 553–567

Sakurada, O., Kennedy, C., Jehle, J., Brown, J. D., Carbin, G. L., Sokoloff, L. (1978) Measurement of local cerebral blood flow with iodo[^{14}C]antipyrine. Am. J. Physiol. 234: H59–H66

Sawada, Y., Sugiyama, Y., Iga, T., Hanano, M. (1987) Tracer distribution kinetics in the determination of local cerebral blood flow by a venous equilibrium model, tube model, and distributed model. J. Cereb. Blood Flow Metab. 7: 433–442

Schinkel, A. H., Smit, J. J. M., Tellingen, O., Beijnen, J. H., Wagenaar, E., Deemter, L., Mol, C. A. M., Valk, M. A., Robanus-Maandag, E. C., Riele, H. P. J., Berns, A. J. M., Brost, P. (1994) Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood–brain barrier and to increased sensitivity to drugs. Cell 77: 491–502

Schinkel, A. H., Wagenaar, E., van Deemter, L., Mol, C. A., Brost, P. (1995) Absence of the mdr1a P-glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin A. J. Clin. Invest. 96: 1698–1705

Shibasaki, J., Konishi, R., Yamasaki, K. (1977) Tolbutamide-sulphaphenazole interaction in rabbits. J. Pharmacokinet. Biopharm. 5: 277–290

Shirai, A., Naito, M., Tatsuta, T., Dong, J., Hanaoka, K., Mikami, K., Oh-hara, T., Tsuruo, T. (1994) Transport of cyclosporin A across the brain capillary endothelial cell monolayer by P-glycoprotein. Biochim. Biophys. Acta 1222: 400–404

Sugita, O., Sawada, Y., Sugiyama, Y., Iga, T., Hanano, M. (1981) Prediction of drug–drug interaction from in vitro plasma protein binding and metabolism. Biochem. Pharmacol. 30: 3347–3354

Sugita, O., Sawada, Y., Sugiyama, Y., Iga, T., Hanano, M. (1982) Physiologically based pharmacokinetics of drug–drug interaction: a study of tolbutamide–sulphonamide interaction in rats. J. Pharmacokinet. Biopharm. 10: 297–316

Takano, M., Hirozane, K., Okamura, M., Takayama, A., Nagai, J., Hori, R. (1994) *p*-Aminohippurate transport in apical and basolateral membranes of the OK kidney epithelial cells. J. Pharmacol. Exp. Ther. 269: 970–975

Tatsuta, T., Naito, M., Oh-hara, T., Sugawara, I., Tsuruo, T. (1992) Functional involvement of P-glycoprotein in the blood–brain barrier. J. Biol. Chem. 267: 20383–20391

Tsuji, A., Terasaki, T., Takabatake, Y., Tenda, Y., Tamai, I., Yamashima, T., Moritani, S., Tsuruo, T., Yamashita, J. (1992) P-Glycoprotein as the drug efflux pump in primary cultures bovine brain capillary endothelial cells. Life Sci. 51: 1427–1437

Yamaoka, K., Tanigawara, Y., Nakagawa, T., Uno, T. (1981) A pharmacokinetic analysis program (MULTI) for the microcomputer. J. Pharmacobiodyn. 4: 879–885