The Use of Intracerebral Microdialysis to Determine Changes in Blood-Brain Barrier Transport Characteristics

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The aim of this study was to determine whether changes in the transport of drugs into the brain could be determined by in vivo intracerebral microdialysis. Atenolol was used as a model drug to determine blood-brain barrier (BBB) transport characteristics. In rats, unilateral opening of the blood-brain barrier was achieved by infusion of hyperosmolar mannitol (25%, w/v) into the left internal carotid artery. BBB transport, expressed as the ratio of the area under the curve (AUC) of atenolol in brain extracellular fluid over plasma, was three times higher for the mannitol treated hemisphere as compared with the contralateral brain or after infusion of saline, being (mean ± SEM) 0.094 ± 0.024 (n = 16), 0.029 ± 0.007 (n = 12) and 0.030 ± 0.009 (n = 12) respectively. Further evaluation of the data indicated that for experiments performed in the morning the mannitol infusion had little effect on the extent of transport of atenolol into the brain, while in the afternoon BBB transport was about 10-fold higher than in the contralateral and saline group. The mean "afternoon" ratios ± SEM were 0.155 ± 0.038 (n = 8), 0.012 ± 0.003 (n = 6) and 0.018 ± 0.006 (n = 6) respectively. It is concluded that intracerebral microdialysis is capable of revealing changes in BBB transport and regional and time-dependent differences in drug levels can be demonstrated with the use of this technique.

KEY WORDS: intracerebral microdialysis; blood-brain barrier; hypertonic opening; atenolol, pharmacokinetics; rat.

INTRODUCTION

Brain endothelial cells differ from peripheral endothelial cells in a number of aspects. Unlike in peripheral endothelium, there are tight junctions between capillary endothelium cells in the brain. Furthermore the cerebral endothelial cells are surrounded by astroglial foot processes, and contain many mitochondria and very few pinocytotic vesicles. These characteristics make the brain capillary endothelium a physical as well as metabolic barrier, called the blood-brain barrier (BBB). It restricts many molecules from entering the brain, while metabolic needs like sugars and amino acids are supplied by carrier-mediated transport mechanisms (1). Most drugs enter the brain by passive diffusion in which the physico-chemical characteristics of a molecule play an important role as well. It has been found that lipid solubility and molecular size are main determinants of the BBB transport of many drugs (2).

In many pathological states (e.g. tumors, inflammation, trauma) the BBB alters markedly both functionally and physically (3,4,5,6). In such cases, an increased penetration of drugs that are normally restricted in their transport into the brain, is mostly found. Also, experimental strategies can be applied to manipulate BBB permeability, mostly aiming at increased concentrations of drugs in the brain. Altered BBB transport, brought about either by a disease state or by an experimental strategy, may be measured in the brain itself by means of an appropriate technique.

If performed under carefully controlled conditions, intracerebral microdialysis offers a useful tool to monitor drug concentrations in a selected area in the brain (7,8). However, the use of this technique in the determination of BBB transport characteristics of drugs should be critically evaluated, for it is an invasive technique by which BBB functionality may be influenced. In a previous study, the BBB transport of two drugs with different lipophilicity was determined with this technique, and it seemed that normal BBB transport characteristics were reflected (27).

In this study, specific attention is paid to the ability of this technique to determine changes in BBB transport of the hydrophilic drug atenolol, for which brain entry is known to be low. If changes in the transport of this drug are in the same order of magnitude as detected by other techniques, including non-invasive ones, it strongly indicates that the use of this technique in such studies is justified. A well-established method of increasing the permeability of the BBB is a brief unilateral intracarotid infusion of a hyperosmolar mannitol solution (25 %, w/v) (9,10,11,12). This apparently opens tight junctions. In this study, the hyperosmolar mannitol solution was infused into the left carotid artery to induce a BBB disruption in the left hemisphere of the brain. Atenolol was measured in the rat left or right cortical brain, following intravenous administration. Serial blood sampling allowed the determination of its profile in blood. The ratio of $AUC_{brain\ ECF}/AUC_{plasma}$ was determined for each individual rat, as a measure of BBB transport, and used to compare the results.

MATERIALS AND METHODS

Animals. Adult male SPF Wistar rats (body weight 235-260 g) were obtained from the Sylvius Laboratories breeding facilities and maintained on a standard laboratory rat diet (RMH-TH, Hope Farms, Woerden) and used throughout the study.

Surgery. The rats were anesthetized with an intramuscular injection of 1 ml/kg of Hypnorm® (Janssen Pharmaceutica, Goirle, The Netherlands) and placed in a stereotaxic frame. Incisions were made to expose the skull which was thereafter locally anesthetized with a 0.6 % solution of lidocaine. A dialysis probe was used which consisted of a dialysis membrane (O.D. 0.29 mm, C-DAK artificial kidney 201-800 D 135 SCE, CD Medical B.V., Rotterdam, The Netherlands), partially covered by silicon glue (Rhodosil CAF 3, RhonePoulenc, Amstelveen, The Netherlands) to leave a 3 mm zone free for dialysis, and a tungsten wire

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within the membrane, to allow a straight introduction of the dialysis probe. A hole of 1.5 mm was drilled in each of the lateral planes of the skull, and at a level of 2.1 mm below the bregma point the dialysis probe was transversally introduced in the brain cortex (TW5-3, Clark Electro Medical Instr., England). The selected dialysis zone of 3 mm was positioned either in the middle of the left or right side of the total width of the intracerebral part of the probe (12 mm). After introduction of the probe, the tungsten wire was removed, leaving the dialysis membrane behind. Stainless steel needles, glued to both ends of the dialysis fibre, were secured with dental cement (Austenal Dental, England) on the top of the skull. The animals were allowed to recover from probe implantation and anesthesia for 25-29 hours before the start of the experiment.

A perfusate temperature of 38°C was achieved by inserting a subcutaneous cannula (polyethylene tube, I.D. 0.58 mm, length ± 20 cm) into the back of the rat. The perfusate fluid was led through this cannula just before entering the microdialysis probe in the brain, allowing the fluid to equilibrate at rat body temperature.

For the intracarotid infusion of hypertonic mannitol the rats were anesthetized with diethyl ether and the left external carotid artery was exposed; then split offs of small arteries were ligated. A polyethylene cannula was inserted up to the bifurcation of the common carotid artery. This allowed a retrograde infusion into the left internal carotid artery with only temporary interruption of the bloodstream in the internal artery. The cannula was made of two pieces of polythene tubing respectively with lengths of 0.3 cm (I.D. 0.28 mm, O.D. 0.61 mm) and 10 cm (I.D. 0.58 mm, O.D. 0.96 mm)

Finally, a polyethylene cannula was implanted into the femoral artery and a polyvinylchloride cannula into the femoral vein respectively, for serial blood sampling and intravenous (IV) drug administration. After this surgery the animals were allowed to recover for two hours before the experiment was started.

Experimental Procedure. For transcortical microdialysis, the stainless steel needles at both sides of the microdialysis probe were connected by means of polyethylene tubing (O.D. 0.61 mm, I.D. 0.28 mm, 80 cm) to a perfusion pump (Gilson) and to a sample loop connected to HPLC. The dialysis probe was perfused at 7 µl/min with a 2 mM phosphate buffer containing 145 mm sodium, 2.7 mM potassium, 1.2 mm calcium, 150 mM chloride, 1.0 mM magnesium (all as ions), and 0.2 mM ascorbate, pH = 7.4. The rats were dialyzed with buffer solution for 30 minutes to obtain dialysis equilibrium and blank data.

After 15 minutes of collecting blank brain dialysate the animals were anesthetized with an intraperitoneal injection of 0.6 ml/kg Nembutal® (Compagnie Rousselot, Paris, France). At the end of equilibrium dialysis the hypertonic mannitol (Sigma, U.S.A.) solution (25 % (w/v) in a 1 mM sodium phosphate buffer pH = 7.4, 38°C) or the control saline solution (in 1 mM sodium phosphate, pH = 7.4, 38°C) was manually infused (3.6 ml/30 sec) over a filter (0.2 µm filter, Schleicher & Schuell, Germany) using a syringe.

After 0.5 minutes a solution of 10 mg of atenolol in 500 µl saline was injected into the femoral vein during 1 minute. Atenolol levels were measured in the dialysate for 120 min. At regular intervals (0, 5, 15, 30, 45, 60, 90, and 120 min) blood samples of 200 µl were drawn from the femoral artery and heparinized. Plasma was obtained by centrifugation and stored at −20°C until analysis.

This study consisted of three experiments. Hyperosmolar mannitol was infused into the left carotid artery and BBB transport of atenolol was measured in the left cortex (ML, n = 16) and in the right cortex (MR, n = 12). The third experiment involved the infusion of isotonic saline into the left carotid while BBB transport of atenolol was measured in the left cortex (SL, n = 12).

Drug Analysis. The atenolol HPLC system consisted of a reversed phase column (Spherisorb, 10 cm * 2 mm I.D., S3 ODS 2, Phase Separations, Waddinxveen, The Netherlands), a precolumn (pellicular reverse phase, Chrompack, Middelburg, The Netherlands) and a Shimadzu RF-350 Fluorimeter (Shimadzu Corp., Kyoto, Japan) with an excitation wavelength of 276 and an emission wavelength of 309 nm. The mobile phase consisted of 73.5 % (v/v) 0.1 M sodium acetate buffer pH = 4.0 containing 5 mM of sodium octane sulfonate and 26.5 % (v/v) of acetonitrile. The flow was 0.2 ml/min.

The microdialysate was injected on-line up to 120 minutes after administration of the drug, into the HPLC system by a Valco injection valve equipped with a 16 µl loop, with a repetition time of 4.0 min. The inter-assay variation in academic solution was 7 % (n = 18), with a detection limit of 30 ng/ml (3 pmol). All assay calibration curves had a correlation coefficient > 0.990).

The assay of plasma was as follows. To 100 µl of plasma of 100 µl of pindolol solution (25 µg/ml water) was added as internal standard. Furthermore, 100 µl of 4M sodium hydroxide and 800 µl of water were added to the samples. Subsequently the aqueous mixture was extracted with 5 ml of ethyl acetate. After cenrifugation (10 min, 4000 rpm) the aqueous layer was discarded and 200 µl of 1 % (w/v) phosphoric acid was added to the organic layer; tubes were shaken vigorously for 30 sec. The layers were separated by centrifugation (10 min, 4000 rpm) and the organic layer was removed. The samples were put in a vacuum evaporator (Vacuum vortex, Buchler, Ford Lee N.Y., U.S.A.) at 30°C for 15 minutes in order to remove ethyl acetate traces from the aqueous layer. The samples were diluted with 200 µl of water and 100 µl was injected into the HPLC system by means of an auto-injector (Wisp 710B, Waters, The Netherlands).

Data Analysis. The concentration-time profiles of the drugs in dialysate were corrected for in vitro recovery of 3.1 % (±0.3 %, SEM) at 38°C, as measured for a 3 mm dialysis zone in a non-stirred solution of atenolol in the perfusate medium. From the $C_{brain\ ECF}$ and C_{plasma} data individual AUC values (0-120 min) were calculated by the trapezoidal rule.

Statistical analysis of the data has been performed with the Student's T-test, the Kruskall-Wallis test or the Wilcoxon rank-sum test ($P < 0.05$).

RESULTS

The hyperosmolar mannitol solution was infused into the left carotid artery to induce a BBB disruption in the left hemisphere of the brain. With intracerebral microdialysis the

$C_{brain\ ECF}$ of atenolol was determined in the left cortex (ML, n = 16) or in the right cortex (MR, n = 12) to investigate whether regional differences in BBB transport could be measured. The control study involved the infusion of isotonic saline into the left carotid artery and BBB transport of atenolol was measured in the left cortex (SL, n = 12). The mean C_{plasma} of atenolol after infusion of mannitol or saline are shown in figure 1. It is clear that mannitol infusion did not affect plasma kinetics of atenolol. For each individual rat the ratio of $AUC_{brain\ ECF}/AUC_{plasma}$ was calculated to indicate the relative BBB transport of atenolol. This ratio was 0.094 ± 0.024, 0.030 ± 0.009 and 0.029 ± 0.007 for ML, SL and MR respectively (fig 2).

Interestingly, it appeared that the time of the day on which the experiment was performed, had a significant effect on the experimental results (figure 3). In the "morning" experiments (started at ± 11.00 hr), no differences in atenolol transport into the brain could be detected between the ML, MR and SL experiments. However, in the afternoon (started at ± 14.30 hr) the results were much different; after mannitol infusion the transport of atenolol into the ipsilateral brain (ML) was augmented by about a factor 10 (fig 3). Also for the saline study a significant time of day effect was seen. In this case a decrease was found (4-fold) between "afternoon" as compared with "morning" experiments. No differences in the AUC_{plasma} were found in "morning" versus "afternoon" experiments (table I).

The post microdialysis surgery interval (PSI) was investigated for its effect on the results. Table II shows that the PSI (hours) could not account for the difference in "morning" versus "afternoon" data.

DISCUSSION

The purpose of this study was to investigate the utility of intracerebral microdialysis for detecting changes in BBB transport of drugs. The results clearly indicate that this technique is capable of detecting (regional) changes in BBB transport of the hydrophilic model drug atenolol, and is therefore considered to be very useful in studying in vivo alterations in BBB transport in general.

The technique of hyperosmotic infusion was used to increase BBB permeability. The unilateral changes in transport of a variety of drugs into the brain after the intracarotid infusion of mannitol into one of the internal carotid arteries

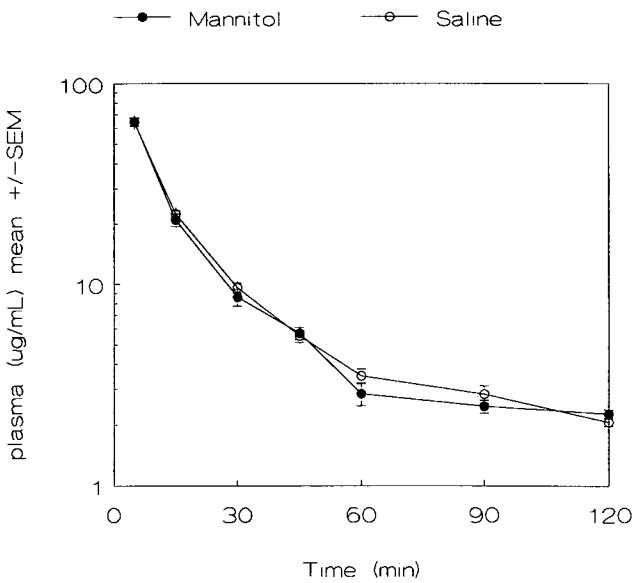

Figure 1 Mean plasma concentration-time profiles (values ± SEM) as obtained after an intravenous bolus injection of 10 mg atenolol, subsequent to mannitol (●, n = 16) or saline (○, n = 12) infusion.

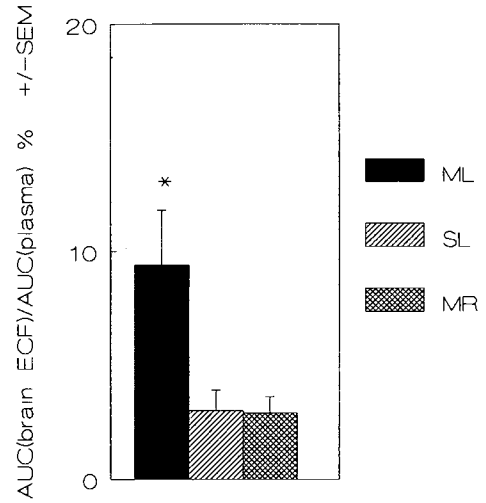

Figure 2 $AUC_{brain\ ECF}/AUC_{plasma}$ ± SEM values of atenolol, after an iv bolus injection of 10 mg, as obtained in the left (ML, n = 16) or right (MR, n = 12) brain cortex subsequently to infusion of hyperosmolar mannitol into the left internal carotid artery; or in the left cortex after saline infusion into the left internal carotid artery (SL, n = 12). * = sign. different (P < 0.05, Wilcoxon rank-sum test) from values of SL and MR.

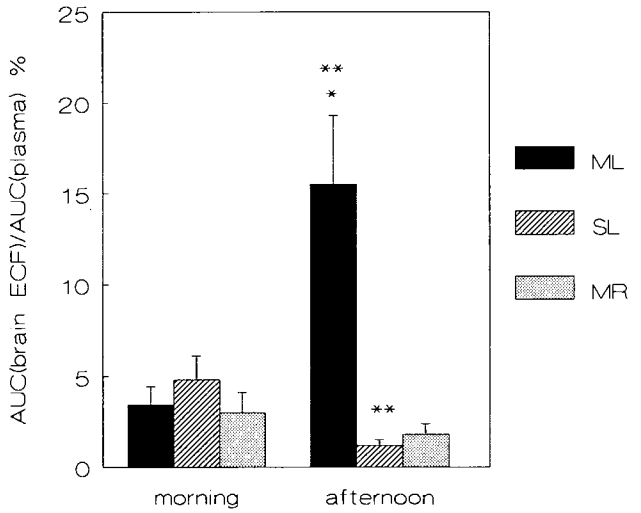

Figure 3 $AUC_{brain\ ECF}/AUC_{plasma}$ ± SEM values of atenolol after iv administration of 10 mg atenolol as obtained in the left (ML, n = 8) or right (MR, n = 6) brain cortex following infusion of hyperosmolar mannitol into the left internal carotid artery; or in the left cortex after saline infusion into the left internal carotid artery (SL, n = 6); difference between morning (n = 8, 6 and 6 respectively) and afternoon experiments (n = 8, 6 and 6 respectively). * = sign. different (P < 0.05, Kruskall Wallis test) from values of SL and MR. ** = sign. different (P < 0.05, Kruskall Wallis test) from corresponding morning values.

Table I. AUC_{plasma} ± SEM Values of Atenolol After iv Bolus Injection of 10 mg, Subsequent to Mannitol or Saline Infusion, in "Morning" and "Afternoon" Experiments

Experiment	AUC_{plasma} (μg · min/ml)
Mannitol morning (n = 14)	1595 ± 131
Mannitol afternoon (n = 14)	1458 ± 72
Saline morning (n = 6)	1561 ± 112
Saline afternoon (n = 6)	1413 ± 58

Values are presented as mean ± SEM (Student's t-test, n.s. for $P < 0.05$).

has been reported by several investigators (11,13,14,15, 16,17). In the present study this method was used to validate intracerebral microdialysis with respect to its ability to assess changes in BBB transport of the hydrophilic drug atenolol. In previous studies (19,27) it had already been demonstrated that atenolol is restricted in its transport into the brain. It is therefore a sensitive means to probe changes in BBB transport.

After the infusion of the hypertonic mannitol solution into the left intracarotid artery, a bolus injection of 10 mg atenolol was administered intravenously, and the resulting concentration-time profiles were obtained in the left (ML, n = 16) as well as in the right (MR, n = 12) cortical brain ECF. It was shown that at the site of infusion (ML) the BBB transport of atenolol, as based on the ratio of $AUC_{brain\ ECF}/AUC_{plasma}$, was three times higher than at the opposite cortex (MR). This means that unilateral alterations in BBB transport by hyperosmolar BBB modification can be detected by this technique. After infusion of saline into the left hemisphere the results obtained from the left cortex (SL, n = 12) were equal to those of the MR (fig 2), as well as to the $AUC_{brain\ ECF}/AUC_{plasma}$ ratios that were obtained after iv atenolol administration alone (3.8 %; 27). This indicates that significant influences of infusion of saline or the presence of mannitol in blood on BBB permeability can be excluded.

After careful examination of the results, it was surprising to find that the effect of the hyperosmotic mannitol could only be demonstrated when the experiments were performed in the afternoon. At that time of day a 10-fold increase in brain concentrations of atenolol was found at the side of hyperosmotic BBB disruption by mannitol (ML) (fig 3). This increase is in the same order of magnitude as the increase reported by Agon et al. (1991) (18), who used a non-invasive technique (PET) to detect atenolol in brains of in monkeys. In the present study, a significant time effect was seen for SL also. When the experimental results obtained in the afternoon were compared with those obtained in the morning, the brain concentrations of atenolol were significantly decreased (fig 3).

Table II. Relationship Between Timing of the Post Microdialysis Surgery Interval (PSI) and the Effect of Hyperosmolar Mannitol Infusion on BBB Transport of Atenolol after iv Bolus Injection of 10 mg

Microdialysis surgery	Experiment	PSI (hr)	$AUC_{brain\ BCI}/AUC_{plasma}$
morning n = 8	morning	25	0.034 ± 0.010
morning n = 8	afternoon	28–29	0.155 ± 0.038*
afternoon n = 3	afternoon	25	0.118 ± 0.031*

Values are presented as mean ± SEM. * = sign. diff. (Kruskall Wallis test, $P < 0.05$) from the values obtained in the morning with PSI of 25 hours.

This time-dependency has not been reported before. As no differences in AUC_{plasma} of atenolol could be found between morning and afternoon for both mannitol and saline (table I), differences in brain concentrations of atenolol should be due to differences in BBB transport. Therefore, the question arose as to whether this is a consequence of the use of the intracerebral microdialysis technique, i.e. the use of multiple anaesthesia in the present experimental design, possible interactions between periprobe tissue reactions with physiological events, or the reflection of "true" time-dependent susceptibility of the BBB to osmotic disruption in a certain area in the brain.

In the current experiments several anesthetic agents were used; fentanyl-fluonisone (insertion of the microdialysis probe); diethyl ether (implantation of cannulas in carotid artery, femoral artery and femoral vein) and pentobarbital (during the experiments). Gumerlock and Neuwelt (15) have shown that anesthetic agents had an effect on hyperosmotic BBB permeability changes, probably by induced cardiovascular changes. The time schedule of administration of the anesthetics in the present experiments was very precise, except for the interval between the implantation of the microdialysis probe and the start of the experiment. This post microdialysis surgery interval (PSI) varied from 25-29 hours. This means that in principle, differences in the remaining effects of fentanyl-fluonisone could exist at the time of the experiment. However, the present data indicated that the PSI did not play a role (table II).

Hence, an interaction of the dialysis technique with physiological events cannot be excluded. The insertion of a microdialysis probe is inevitably traumatic and may result in formation of edema in the periprobe tissue (19). Endogenous compounds that exhibit circadian fluctuations in their levels may have an effect on the pathophysiological processes that follow probe implantation. Without osmotic BBB opening the transport of atenolol was not subjected to time-dependency in microdialysis experiments performed in the morning, afternoon, or evening. However, it cannot be excluded that the periprobe tissue might have different sensitivities to hyperosmotic insults and/or infusion of large volumes at different times of the day.

Alternatively, it is conceivable that the results obtained with intracerebral microdialysis reflect "true" differences in the local changes in BBB transport. It is known that for the osmotic opening of the BBB, a certain threshold product of "osmolarity × infusion time" is critical (10). Due to possible differences in regional cerebral flow resistance during the day (20), not all of the infused volume may be equally distributed over the left brain circulation in the course of the day, and the critical threshold product may not be reached at the site of the dialysis probe in the cortical brain of "morning" experimental animals. It is also possible that the levels of certain compounds within body fluids may exert an effect on BBB susceptibility for osmotic opening. Variability ac-

cording to the time of the day on which the experiment was performed may therefore be caused by differences in the level of these compounds, e.g. by dietary factors or by circadianic rhythms. Endogenous compounds showing a circadian rythm include steroids. For corticosteroids and their agonist dexamethasone an effect on BBB permeability have already been shown (21,22,23,24). An inhibitory effect of dexamethason on hyperosmotic BBB opening has been demonstrated (25,26), however, to date no reports have been made on interaction of dietary factors or endogenous compounds with circadian changes in their levels, and hyperosmotic BBB disruption. So, the reason(s) for the intruiging differences in the effect of hyperosmotic mannitol infusion on BBB permeability between morning and afternoon experiments remain to be clarified, and will be the subject of subsequent studies.

In conclusion it was found that changes in atenolol transport after BBB modification can be assessed by intracerebral microdialysis, which includes the detection of regional differences in drug concentrations. Intracerebral microdialysis is therefore a powerful tool to investigate BBB transport, with applications e.g. in research on strategies to increase drug transport into the brain.

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