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Lidocaine distribution into the CNS following nasal and arterial delivery: a comparison of local sampling and microdialysis techniques

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Abstract

The disposition of lidocaine within the CNS of the rat following nasal and intra-arterial delivery was characterized using a microdialysis technique. Lidocaine concentrations in the cisterna magna were determined using microdialysis and compared to those previously determined using a direct CSF sampling method. The disposition profiles for lidocaine into the cisternal CSF obtained using microdialysis were found to be similar to those obtained by direct CSF sampling techniques over an initial 120-min interval. In other experiments, lidocaine disposition in the right (dosed side) and left olfactory bulb following nasal (i.n.) and intra-arterial (i.a.) administration was studied using microdialysis. The lidocaine concentrations in the ipsilateral olfactory bulb were slightly higher after drug administration into the nasal cavity than those in the contralateral olfactory bulb over the initial 20-min sampling interval. Drug concentrations found in the right olfactory bulb were not significantly different from those found in the left olfactory bulb following intra-arterial administration. Comparisons of lidocaine disposition in the right olfactory bulb and cerebellum, two CNS sites with the same regional vascular supply, showed that the disposition patterns were nearly identical for the two sites following i.a. administration. There was a significant lengthening in the t_{max} at both sites following i.n. delivery compared to i.a. delivery, and the relative concentrations at each site were no longer equivalent. From these results, it appears that the microdialysis technique is a useful tool for studying drug distribution into the CNS. The changes in disposition patterns between i.a. and i.n. administration indicate that other factors or pathways, in addition to the systemic circulation, play a role in the transport of lidocaine into the brain following nasal administration.

Keywords: Intranasal delivery; Lidocaine hydrochloride; Central nervous system; Microdialysis; Olfactory bulb; Cerebellum

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1. Introduction

Intranasal delivery of certain therapeutic agents has been found to result in an enhanced absorption into the cerebrospinal fluid (CSF) (Kumar et al., 1974a,b, 1979, 1982; Sakane et al., 1991a,b, 1994; Frey et al., 1995; Gizurarson et al., 1996; Chou and Donovan, 1997). The elevated drug concentrations in the CSF raise questions regarding the role that the cerebrospinal fluid plays in the transport processes following intranasal administration. A direct transport pathway from the nasal cavity into the CSF is most likely to involve substances being taken up by the olfactory dendrites projecting through the nasal olfactory epithelium then transported either through the extension of subarachnoid space surrounding the olfactory bundle or by axoplasmic flow in the olfactory nerve (Gopinath et al., 1978; Jackson et al., 1979). The resulting appearance of drug in the CSF could be the consequence of distribution via the olfactory transport pathway or merely the cerebrospinal fluid from the subarachnoid space functioning as the transport medium. To elucidate the mechanism as well as the transport pathways from nasal membrane into the CSF, attempts such as characterization of the physicochemical properties controlling the preferential absorption into the CSF (Sakane et al., 1991b, 1994, 1995; Chou and Donovan, 1997) and histologic studies on the movement of marker compounds along the olfactory nerves (Kristensson and Olsson, 1971; Meredith and O'Connell, 1988; Hastings and James, 1991; Evans and Hastings, 1992) have been reported. However, these results only provide indirect evidence regarding the transport pathway. The lack of quantitative studies regarding the distribution of substances within different CNS regions leaves many questions unanswered regarding this unique pathway to the CNS.

To assess drug distribution into the CNS, the ability to continuously monitor the concentration changes in the extracellular space of the brain is essential. However, the limited quantity of the extracellular fluid within the brain tissues makes frequent sample withdrawal impossible. Microdialysis techniques not only allow the sampling of chemical contents of the extracellular fluids (ECF) of the tissues of interest without removal of any body fluids, but they also allow the monitoring of local chemical environments within a small, well-defined area. Therefore, microdialysis could be a valuable tool to characterize the disposition of therapeutic agents into the CNS.

In these studies, microdialysis was used to estimate lidocaine concentrations in different regions of the rat brain following nasal administration compared to those obtained after parenteral administration. Intranasal delivery of lidocaine has been used to treat patients suffering from migraine (Maizels et al., 1996). Rapid and effective relief of pain was reported to occur within 5 min after drug administration for over 50% of the patients. Therefore, lidocaine was selected from a group of local anesthetics previously evaluated for their transport into the CNS following nasal delivery to study disposition from the nasal cavity into and within the brain (Chou and Donovan, 1998).

2. Materials and methods

2.1. Chemicals

Lidocaine hydrochloride was purchased from Pfaltz and Bauer (Stamford, CT). HPLC grade acetonitrile was obtained from EM Science (Gibbstown, NJ). Decanesulfonic acid was purchased from Sigma (St. Louis, MO). Phosphoric acid was obtained from Mallinckrodt (Paris, KY). All chemicals and solvents were used without further purification.

2.2. Microdialysis probes and artificial CSF

Microdialysis probes were obtained from ESA (Bedford, MA). A loop type probe with a cellulose acetate dialysis membrane was used for both the in vitro and in vivo studies. The membrane was 2 mm in length with a molecular weight cut-off of 6000 Da. The tip diameter of the probe was 450 μm. Artificial CSF composed of 128 mM NaCl, 2.6 mM KCl, 1.26 mM $CaCl_2$ and 2 mM $MgCl_2$ was prepared using deionized distilled water. The solution was filtered through a 0.47-μm nylon filter before use.

2.3. In vitro microdialysis

In vitro microdialysis experiments were performed to determine the influence of the external medium surrounding the dialysis probes on the relative recovery of lidocaine. These were accomplished by immersing dialysis probes into a 0.4-μg/ml solution of lidocaine hydrochloride prepared in either artificial CSF or Lactated Ringer's solution. Artificial CSF was perfused through the probes at flow rates of 3 μl/min and 5 μl/min using an infusion pump (Model 22, Harvard Apparatus, South Natick, MA) with a 1-ml gas-tight syringe (Hamilton, Reno, NV). The in vitro microdialysis experiments were carried out at room temperature. Perfusates were collected over 20-min intervals. The outlet tubing was limited to 20 cm to prevent membrane bluffing (expansion) due to high back pressure.

Additional experiments designed to study the effect of flow rate as well as concentration on recovery were conducted. Artificial CSF was perfused through the probes immersed in various concentrations (0.4, 1, 3 μg/ml) of lidocaine hydrochloride prepared in artificial CSF at flow rates between 2 and 8 μl/min. For lidocaine hydrochloride solutions with concentrations of 0.4 and 3 μg/ml, the probes were perfused at each of three flow rates (3, 5, 7 μl/min). The system was allowed to equilibrate for 1 h in blank artificial CSF prior to changing the flow rate or concentration. Samples were collected starting immediately after placing the probe into the drug solution. At each flow rate, four samples were collected over 20-min intervals. To avoid the impact of the relative humidity/temperature conditions in the laboratory at different times of the study on evaporation of sample volume, all in vivo or in vitro samples were collected into a capped 1.5-ml Eppendorff tube with a hole made in the cap which allowed for placement of the outlet tubing into the tube.

2.4. Animal preparation

The animal experiments adhered to the 'Principles of Laboratory Animal Care' (NIH publication # 85-23, revised 1985) and were approved by the University of Iowa Committee on the Use and Care of Animals. Male Sprague–Dawley rats (350–400 g) were given an intramuscular dose of 50% (w/v) urethane (1.5 g/kg), followed by a supplementary dose of a mixture of ketamine (47 mg/kg) and xylazine (7 mg/kg) to obtain full surgical anesthesia. Both femoral arteries of the anesthetized animals were cannulated to allow for drug administration and blood collection. The nasal cavity was isolated from the respiratory and gastrointestinal tracts using a modification of the method of Hussain et al. (1980). Direct samples of CSF were obtained by cannulating the cisterna magna (Chou and Donovan, 1997). The resulting plasma and CSF concentrations are fully described in Chou and Donovan (1998).

2.5. Placement of dialysis probes in vivo

To place the dialysis probes, the head of the anesthetized rat was secured on a stereotaxic frame. The allanto-occipital membrane was exposed by making a blunt dissection through the allanto-occipital muscle. A hole was made in the membrane using a 21G needle to access the cisterna magna. The probe was then placed through the hole. The tip of the probe was inserted 3.5 mm below the membrane so that the entire surface area of the probe was bathed in the CSF. For the animals used to compare lidocaine concentrations between the right and left olfactory bulbs, two holes were drilled bilaterally at the following stereotaxic coordinates: anterior 7 mm and lateral 1.5 mm from the bregma. The tips of the probes were placed 4 mm below the skull (Pellegrino et al., 1979). For the comparison between the olfactory bulb and cerebellum, probes were placed in the right olfactory bulb (as previously described) and the cerebellum. To locate the cerebellum, the stereotaxic coordinates: posterior 8.8 mm and lateral 3.0 mm were used, and the tip of the probe was placed 5.5 mm below the skull (Pellegrino et al., 1979). The implanted probes were perfused with artificial CSF at a flow rate of 3 μl/min for 1 h prior to drug administration and continuously throughout the entire experiment. The perfusate fraction collected during a 20-min interval prior to drug administration was used as

the baseline sample. Each probe was subject to in vitro recovery studies before the in vivo experiments to assure its full functioning and to determine its relative recovery.

2.6. In vivo absorption studies

Each anesthetized animal received a 17-mg/kg dose of lidocaine hydrochloride either intra-arterially or intranasally (Chou and Donovan, 1997). Drug solution was prepared in pH 6.8, 0.1 M Sørensen's phosphate buffer. For intra-arterial administration, 50 μl of drug solution were administered via the femoral artery and blood samples were collected from the contralateral artery. For nasal administration, 50 μl of drug solution were placed into the right nostril by carefully inserting a length of PE-10 tubing attached to a volumetric syringe containing the drug solution.

2.7. Analytical procedures

Lidocaine concentrations in the perfusate were measured by HPLC. Perfusate samples were directly injected without further treatment. The HPLC system consisted of a Spectra Physics SP8700 ternary solvent delivery system, an SPD-6A UV spectrophotometric detector, a CR-6A Chromatopac integrator (Shimadzu, Kyoto, Japan), and an WISP 710B autosampler (Waters Chromatography, Milford, MA). For the separation of lidocaine from other biological materials present in the perfusate, a μBondapak C18 column (Waters Chromatography, Milford, MA) was used, with a mobile phase of 25% acetonitrile in acidified water (pH 3.0) containing 3 mM decanesulfonic acid as an ion-pairing agent. The UV wavelength for detection was 210 nm.

2.8. Data analysis

Results from the HPLC analyses were plotted as corrected (by in vitro recovery) drug concentration from the microdialysate versus time. Drug concentrations measured in the dialysate sample collected over a 20-min interval were treated as the fraction of external concentration achieved at the end of 20 min rather than at the mid-time point of the collection interval. The error caused by the lag-time effect in determining true tissue concentration was minimal and not included in the interval calculation. The AUC values for each curve were calculated from time zero to the last data point using the linear trapezoidal rule. Statistical comparisons between the AUC values were tested using the Student's t-test. The level of significance used was 0.05 unless otherwise indicated in the text.

3. Results

3.1. In vitro microdialysis

The effects of flow rate and concentration surrounding the dialysis probe on the in vitro relative recovery of lidocaine are shown in Fig. 1. The relative recovery was determined as the ratio of lidocaine concentration found in the perfusate to that found in the external medium. It can be seen that relative recovery of lidocaine decreased as the flow rate increased regardless of the drug concentration in the external medium. The recovery values obtained within the flow rate and concentration ranges used in these studies were all below 10%, and it appears that drug concentra-

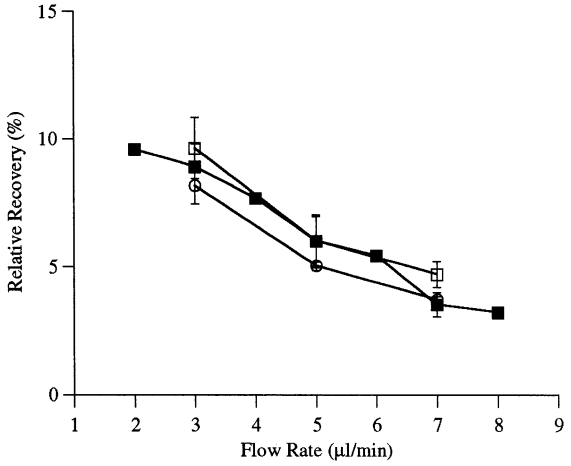

Fig. 1. Effect of flow rate and concentration on relative recovery of lidocaine hydrochloride during microdialysis in vitro (□: 0.4 μg/ml, ■: 1 μg/ml, ○: 3 μg/ml). Data represent the mean ± S.E.M. ($n = 3$).

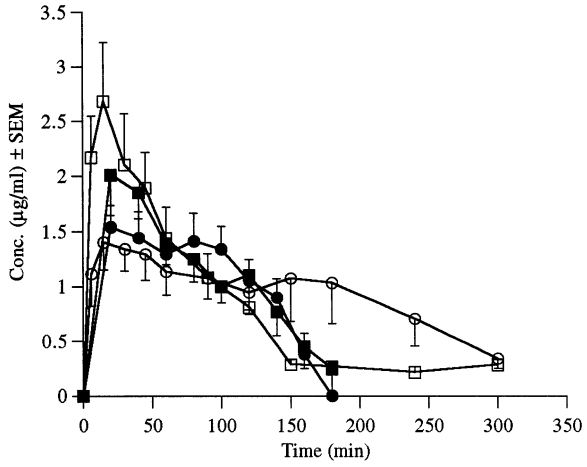

Fig. 2. Lidocaine CSF concentrations following intra-arterial (□, ■) and nasal (○, ●) administration (open symbol: from direct cisterna magna sampling (Chou and Donovan, 1998); closed symbol: from microdialysis). Data represent the mean ± S.E.M. ($n = 3$).

tion had little effect on the relative recovery of lidocaine. The concentrations selected for use in the in vitro recovery experiments were close to the highest, median and lowest concentrations found in CSF samples during previous studies (Chou and Donovan, 1998). It should also be noted that the exchange of lidocaine between the medium surrounding the dialysis probe and the perfusion solution was minimally influenced by the contents of the external medium when artificial CSF was compared to Lactated Ringer's solution. There was no difference in recovery at higher (5 μl/min) flow rates, and a small increase ($p = 0.1$) in recovery in artificial CSF at 3 μl/min.

3.2. In vivo absorption studies

The animals used in these studies were divided into three groups, each containing at least six rats. Each group was further assigned to two subgroups according to the route of administration. For the first group, lidocaine concentrations in the cisterna magna were determined using microdialysis and compared to those obtained using the direct CSF sampling method (Chou and Donovan, 1998). The results are shown in Fig. 2 and summarized in Table 1. Drug concentrations found in the perfusate were corrected by the probe in vitro relative recovery to estimate drug concentrations in the cisternal CSF. The disposition profiles for lidocaine into the CSF obtained using microdialysis were similar to those obtained by directly sampling the CSF. The peak concentrations of lidocaine hydrochloride found in the CSF samples and in the perfusates were achieved within 20 min regardless of the route of administration. However, the disappearance of lidocaine hydrochloride after 120 min from the CSF following nasal administration measured using the microdialysis technique was faster than that found using the direct CSF sampling technique.

In a second group of animals, relative drug concentrations in the olfactory bulb as well as in

Table 1
AUC values for lidocaine distribution into the brain following nasal and intra-arterial (i.a.) administration

Group #	Sampling site	$AUC_{0-180\ min}$ (μg/min per ml)		Ratio of AUC
		i.a.	Nasal	(Nasal/i.a.)
1	Cisternal CSF[a]	202 (17.4)[c]	312 (93.0)[c]	1.54
	Cisterna magna[b]	176 (33.4)	189 (21.2)	1.07
2	Cerebellum	185 (26.1)	129 (17.6)	0.70
	Ipsilateral olfactory bulb	177 (28.9)	158 (20.5)	0.89
3	Ipsilateral olfactory bulb	165 (14.3)	160 (15.4)	0.97
	Contralateral olfactory bulb	176 (16.9)	143 (16.1)	0.81

Values in parenthesis represent ± S.E.M.
[a] Determined by direct CSF sampling technique
[b] Determined by microdialysis technique
[c] 0–300 min.

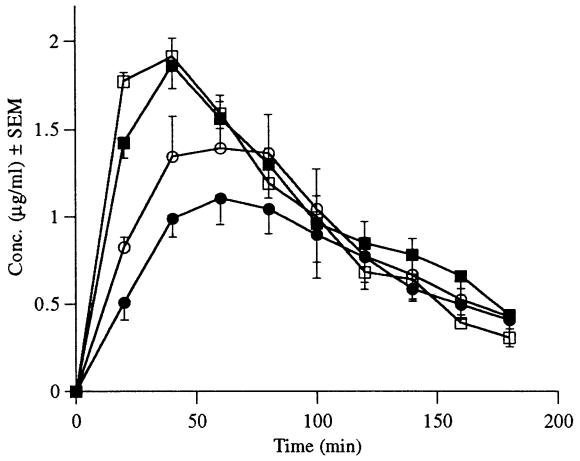

Fig. 3. Lidocaine brain ECF concentrations in cerebellum and right olfactory bulb following intra-arterial (□, ■) and nasal (○, ●) administration (open symbol: right olfactory bulb; closed symbol: cerebellum). Data represent the mean ± S.E.M. ($n = 3$).

the cerebellum were determined simultaneously (Fig. 3, Table 1). These sites were compared since the regional cerebral blood flow to each site has the same origin. Differences in the rate of appearance of lidocaine at either site would suggest the presence of an extra-vascular transport pathway at that tissue site. Differences in tissue concentrations in the cerebellum and the right olfactory bulb were observed following nasal administration, yet differences were not observed following intra-arterial administration. The t_{max} values for lidocaine appearing in the olfactory bulb and cerebellum after nasal administration were equal and longer than those obtained after intra-arterial administration. Following intra-arterial administration, the t_{max} values in the olfactory bulb were also found to be equal to those measured in the cerebellum following intra-arterial administration. The t_{max} values in the cisterna magna were shorter than those in the olfactory bulb or the cerebellum regardless of route of administration.

The rank order of the ratios of $AUC_{i.n.}/AUC_{i.a.}$ were: cisterna magna > olfactory bulb > cerebellum, with the cisterna magna ratio > 1 and the other ratios < 1 (Table 1). Since the systemic bioavailability of lidocaine following nasal administration is 100% (Chou and Donovan, 1998), these AUC ratios would be expected to be equal to 1 if drug disposition at each of the sites was entirely determined by the plasma concentration. This observation, together with higher drug levels found in the ipsilateral olfactory bulb following intranasal administration indicate that additional mechanisms are involved in the disposition of substances into the CNS from the nasal cavity.

Fig. 4 shows the lidocaine concentrations in the right and left olfactory bulbs after nasal and intra-arterial administration that were measured in a third group of animals. Lidocaine was administered either via the right femoral artery or right nostril of the animal. Drug concentrations found in the ipsilateral olfactory bulb were not significantly different from those found in the contralateral olfactory bulb following intra-arterial administration. However, lidocaine levels in the right olfactory bulb during the initial 20 min following drug administration into the nasal cavity were significantly higher than those in the left olfactory bulb. It can also be seen that the distribution of lidocaine into the olfactory bulbs was slower and the peak relative concentrations were lower after nasal administration than after par-

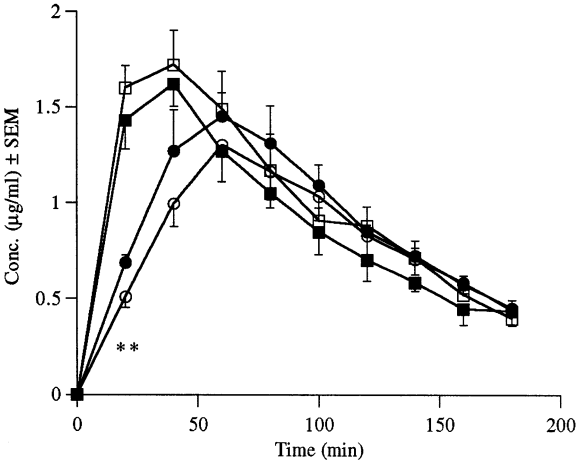

Fig. 4. Lidocaine brain ECF concentrations in right and left olfactory bulb following intra-arterial (□, ■) and nasal (○, ●) administration (open symbol: left olfactory bulb; closed symbol: right olfactory bulb). Data represent the mean ± S.E.M. ($n = 3$). **Significant difference found between ipsilateral and contralateral olfactory bulb following nasal administration ($p < 0.05$).

enteral administration regardless of which olfactory bulb was sampled.

4. Discussion

The application of microdialysis techniques for monitoring the concentration changes in different areas of the body is based on the assumption that the relative recovery from the probe is not strongly dependent on the external environment surrounding the probe. This assumption was shown to be valid in vitro for these studies. Since the concentration ranges selected for study were based on lidocaine levels in the CSF found in our previous studies, along with the observation that the content of the external fluid surrounding the probes had no significant effect on the recovery of lidocaine, it appears that the microdialysis technique is a suitable method for studying the disposition of lidocaine into the CNS following nasal and intra-arterial administration.

To determine true tissue concentration based on the knowledge of dialysate concentration, several methods have been proposed by previous investigators. Among them, the flow-rate (Jacobson et al., 1985; Stahle et al., 1990) and the zero-net flux method (Lonnroth et al., 1987; Stahle et al., 1990) do not appear to be suitable for studies carried out with this anesthetized animal model due to the extended experimental period required by these techniques. The retrodialysis method (Wang et al., 1991; Wong et al., 1992), which assesses in vivo recovery by introducing a calibrator with comparable diffusion characteristics as the solute being studied into the perfusate avoids these long study periods. However, while the transport mechanisms from the nasal membrane to the CNS are still unclear, the use of a calibrator with similar physicochemical and pharmacologic properties as the compound of interest may complicate the interpretation of any transport pathway results. Therefore, in these studies, lidocaine concentrations in the selected CNS compartments were estimated by correcting the dialysate concentrations with the relative recovery determined in vitro. These results may significantly underestimate true drug concentrations achieved in a tissue due to rapid tissue clearance in a particular compartment (Hammarlund-Udenaes et al., 1997) or due to the differences between the in vitro and in vivo diffusional behavior of drug compounds. However, the similarities found in the disposition profiles determined using microdialysis and a direct CSF sampling method indicate that the in vitro recovery of lidocaine was a good indicator of its in vivo recovery in the cisterna magna.

The tissue concentrations in the cerebellum and olfactory bulbs calculated using the in vitro recovery correction may be significantly different from the actual tissue concentrations, but comparisons of the relative concentrations at each tissue site as a result of a change in site of drug delivery are still valid since the probe recovery would not be expected to be affected by administration route. Quantitative comparisons of ratios of the AUC values at the individual tissue sites calculated from the concentration-time profiles are also warranted, even in the light of uncertainty in the recovery factor, since the same factor would have been applied to both aspects of the ratio. Finally, comparisons between the t_{max} values are also valid even when the recovery factor is uncertain, since regardless of the actual concentration value, the t_{max} remains unchanged.

The regional cerebral blood supplies for the olfactory bulb are nearly identical to those for the cerebellum (Rapoport et al., 1979). This may explain the comparable relative concentrations of lidocaine found in the olfactory bulb and cerebellum after intra-arterial administration. Similarly, Czerniawska (1970) studied the transport of gold into the brain area and found that gold levels in the CSF and olfactory bulb were significantly higher than those in the cerebellum following nasal administration. Frey et al. (1995) also reported that intranasal delivery of nerve growth factor resulted in much lower levels in the cerebellum compared to the levels in the olfactory bulb. It appears that the olfactory pathway plays a significant role in the transport of certain substances into the brain from the nasal cavity. There is anatomic evidence that the olfactory bundles are surrounded by an extension of the subarachnoid space as they pass through the cribriform

plate into the nasal cavity. It has also been proposed that substances could be transported by the CSF surrounding the olfactory bundles (Jackson et al., 1979; Bradbury and Westrop, 1983). Transport via this pathway may be responsible for the higher concentrations of lidocaine, gold and nerve growth factor found in the olfactory bulb than in the cerebellum. Preferential transport of lidocaine via the ipsilateral olfactory bulb (Fig. 4) also provides supportive evidence for the existence of this pathway. However, Hussain et al. (1990) found that the direct transport pathway from the nasal epithelium into the brain may be significant only for poorly absorbed compounds. For well absorbed compounds, the olfactory pathway is relatively slow and insignificant due to rapid absorption into the systemic circulation. Lidocaine is a relatively lipophilic compound with a log PC value of approximately 2. As a result, good absorption of lidocaine into the systemic circulation would be expected. However, the results of these studies demonstrate that the olfactory pathway, in addition to the systemic circulation, contributes, though of lower magnitude, to the transport of lidocaine into the brain following nasal administration.

Seki et al. (1994) reported that zidovudine (AZT) concentration found in the initial fraction of CSF collected 15 min after nasal administration of the drug was much lower than that found in later discrete CSF samples. The difference in the CSF concentrations of AZT between the first and last fraction following intravenous infusion was not statistically significant, however. They concluded that a direct transport pathway exists from the nasal cavity into the CSF based on the assumption that the final fraction represented the CSF in the vicinity of nasal cavity. Their studies demonstrated that there may be concentration gradients for drug distributing within the CSF after nasal administration. It is possible that the difference in the rate of disappearance of lidocaine from the CSF and from the perfusate after nasal administration (Fig. 2) may be due to a similar concentration gradient existing in the CSF for lidocaine. The direct CSF sampling method involves the withdrawal of the CSF from the bulk volume circulating through the entire CNS and the constant infusion of replacement CSF into the ventricles. The microdialysis technique only monitors the chemical changes in the local region. The concentrations measured in the cisterna magna locally may be different than those measured in the bulk CSF when concentration differences exist along the CSF circulation pathway. This effect could explain the observation of a more rapid disappearance of lidocaine from the microdialysate in an undisturbed cisterna magna than from the discrete CSF sampled under abnormal flow conditions.

In conclusion, microdialysis appears to be a useful tool in studying drug distribution into the CNS following different routes of drug administration. This technique allows the detailed investigation of the disposition of lidocaine from the nasal cavity into the CNS, and these studies have demonstrated that the nasal disposition pattern does not entirely mimic the disposition pattern seen after parenteral administration. These patterns have demonstrated that it is likely that there are mechanisms involved in the transport of lidocaine into the CNS from the nasal cavity in addition to the systemic circulation. Thus, the nasal cavity might provide an alternative route for central nervous system (CNS) targeted drug delivery, especially for poorly absorbed compounds.

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