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Epinephrine Is Metabolized by the Spinal Meninges of Monkeys and Pigs

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Background: **Epinephrine commonly is added to epidural opioids and local anesthetics, however, little is known about the fate of epidurally administered epinephrine. Studies have identified the epinephrine metabolizing enzyme, catechol-O-methyl transferase (COMT), in the cranial meninges of several species. The purpose of this study was to determine whether the spinal meninges also contain COMT and are capable of metabolizing epinephrine. If so, then the spinal meninges may have an important impact in limiting the bioavailability of epinephrine in both the spinal cord and epidural space.**

Methods: **Spinal meningeal specimens measuring 4 cm^2 were obtained from monkeys (*M. nemestrina*) and farm-bred pigs and were incubated in bicarbonate-buffered mock cerebrospinal fluid. Epinephrine (200 μg base) was added at t = 0, and 200 min later, the mock cerebrospinal fluid was collected for metanephrine analysis. In separate experiments, pig meningeal specimens were separated into dura mater, pia-arachnoid mater, and pia mater, and the experiments were repeated to determine which meninx had the greatest COMT activity.**

Results: **Metanephrine was produced by monkey meninges at the rate of 0.47 $ng \cdot min^{-1} \cdot cm^{-2}$ and by pig meninges at the rate of 0.23 $ng \cdot min^{-1} \cdot cm^{-2}$ ($P > 0.05$). The pia-arachnoid meninx produced metanephrine at a greater rate (4.48 ± 0.46 $ng \cdot min^{-1} \cdot mg^{-1}$ tissue) than did the pia mater (1.3 ± 0.15 $ng \cdot min^{-1} \cdot mg^{-1}$ tissue) or dura mater alone (1.82 ± 0.23 $ng \cdot min^{-1} \cdot mg^{-1}$ tissue).**

Conclusions: **These data demonstrate the functional presence of COMT in the spinal meninges of pigs and monkeys and suggest that the spinal meninges may limit the spinal bioavailability of epidurally or intrathecally administered epinephrine. (Key words: Drug metabolism: catechol-O-methyl transferase; metanephrine. Monkey: *M. nemestrina*. Pig. Spinal cord, meninges: arachnoid mater; dura mater; pia mater. Sympathetic nervous system, catecholamines: epinephrine.)**

EPINEPHRINE commonly is added to epidural opioids and local anesthetics and has been shown to prolong the duration and improve the quality of epidural anesthesia and analgesia.[1–4] However, the mechanism by which epinephrine exerts these effects is unclear. Conventional wisdom has suggested that epinephrine's beneficial effects result from local vasoconstriction and a consequent reduction in drug clearance from the epidural space.[5–8] However, epinephrine produces analgesia when administered into the subarachnoid space by stimulating α_2 adrenoreceptors in the spinal cord dorsal horn.[9–11] Thus, it is possible that epidural epinephrine prolongs the duration and improves the quality of analgesia from epidural opioids and local anesthetics by a direct analgesic effect within the spinal cord dorsal horn.

However, to produce analgesia within the spinal cord, epidurally administered epinephrine must cross the spinal meninges intact. Recent studies have identified catechol-O-methyl transferase (COMT), the enzyme that metabolizes epinephrine to metanephrine, in the cranial meninges of several mammalian species, although these studies have not investigated whether epinephrine is metabolized by the cranial meninges.[12,13] If COMT is also present in the spinal meninges, it is possible that epidurally administered epinephrine is metabolized as it diffuses through the spinal meninges *en route* to the spinal cord. Metabolism of epinephrine by the spinal meninges would be expected to limit the spinal bioavailability of epidurally administered epinephrine. However, the presence of COMT in the spinal meninges and the ability of the spinal meninges to metabolize epinephrine has not been investigated.

The purpose of this study was to determine whether the spinal meninges are capable of metabolizing epinephrine, and if so, to identify which meninx has the greatest metabolic activity. To study this question, we

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used a simple *in vitro* model to quantitate the metabolism of epinephrine by monkey (*M. nemestrina*) and pig spinal meninges.

Materials and Methods

Studies were approved by the University of Washington Animal Care Committee, and guidelines of the American Association for Advancement of Laboratory Animal Care were followed throughout.

Tissue Preparation

Monkey tissues were obtained from animals scheduled to be killed as part of the tissue distribution program of the University of Washington Regional Primate Research Center. Pig tissues were obtained from farm-bred pigs. All meningeal specimens were removed from animals anesthetized with thiopental and ketamine (monkeys) or halothane in 60% N_2O and oxygen (pigs).

The spinal cords of *M. nemestrina* monkeys and farm-bred pigs were exposed from T5 to L5 by laminectomy. The spinal cord was removed *en bloc*, and all three meningeal layers were carefully reflected from the spinal cord, preserving their normal anatomic relationships. From this sheet of intact meningeal tissues, specimens measuring approximately 4 cm^2 were cut for the experiments described below.

Control Experiments

Because epinephrine may undergo spontaneous oxidation in air, we performed control experiments to demonstrate the chemical stability of epinephrine over time in our preparation. For these control experiments, 200 μg epinephrine base as the bitartrate salt (Sigma, St. Louis, MO) was added to temperature-controlled (37°C) test tubes containing 10 ml of bicarbonate-buffered mock cerebrospinal fluid (CSF; NaCl 140 mEq, $NaHCO_3$ 25 mEq, KCl 2.9 mEq, $MgCl_2$ 0.4 mEq, urea 3.5 mEq, glucose 4.0 mEq, $CaCl_2$ 2.0 mEq; *p*H 7.38–7.42; 292–298 mOsm) and bubbled with air (95%) and carbon dioxide (5%). Ascorbic acid (1 mM) was added to prevent spontaneous oxidation of epinephrine.[14] After addition of epinephrine, 500-μl samples were collected every 20 min for 200 min. Perchloric acid (200 μl of 0.1 M) was added to acidify each sample and thereby prevent autooxidation of epinephrine. The samples were capped immediately to minimize further air contact and frozen at −20°C until analyzed for epinephrine.

To demonstrate that epinephrine metabolites were not produced by the meningeal tissues in the absence of exogenous epinephrine, four monkey tissue specimens of intact dura-arachnoid-pia mater measuring 4 cm^2 were incubated in 10 ml of mock CSF containing 1 mM ascorbic acid in the absence of exogenous epinephrine. After 200 min, the 10 ml of mock CSF was collected and acidified with perchloric acid. The samples were frozen at −20°C until analyzed for epinephrine metabolites.

Epinephrine Metabolism Experiments

After demonstrating that epinephrine was stable over time in our model, we repeated the procedures as described above for the control experiments, except that a meningeal tissue specimen measuring approximately 4 cm^2 was placed in the test tube and equilibrated to 37°C before adding 200 μg of epinephrine base. The tissue specimens used for these experiments consisted of intact dura-arachnoid-pia mater (monkey n = 6, pig n = 6), of dura mater alone (pig n = 5), of pia mater alone (pig n = 5), or of pia-arachnoid mater (pig n = 5). After 200 min, the 10 ml of mock CSF was removed, and 1.5 ml of perchloric acid (0.1 M) was added to acidify the sample. Samples were frozen at −20°C until analyzed for metanephrine, normetanephrine, 3-methoxytyramine, dopamine, and norepinephrine. At the completion of the experiment, the pig meningeal specimens were blotted dry and weighed.

Chemical Analysis

The stability of epinephrine over time in the control experiments was confirmed by HPLC assay, using a method modified from Ganhao *et al.*[15] Briefly, this method involves addition of 3,4-dihydroxybenzylamine HBr as an internal standard (Sigma). Epinephrine was separated by HPLC using a BAS Phase II column (Bioanalytical Systems, West Lafayette, IN) and detected by an LC-4A electrochemical detector (Bioanalytical Systems). This assay has a coefficient of variation of <7%, and the limits of quantification are 1–11 μg/ml.

Catecholamine metabolite concentrations (metanephrine, normetanephrine, 3-methoxytyramine) were determined using a modification of the method of Jouve *et al.*[16] (Bio-Rad instruction manual 195-6606, Bio-Rad Laboratories Clinical Division, Hercules, CA). Briefly, 4-O-methyldopamine was added as an internal standard, and the samples were separated by HPLC using isocratic separation (Waters 590 Pump and Waters 745 B Data Module, Millipore, Milford, MA). Metabo-

lites were detected by an electrochemical detector (Coulochem model 5100 A and model 5011 Analytical Cell, ESA, Bedford, MA). The limits of quantification for metanephrine, normetanephrine, and 3-methoxytyramine was 10 ng/ml with a coefficient of variation of 10% at a concentration of 100 ng/ml and 7% at a concentration of 500 ng/ml.

Statistical analysis

Differences between the species in amount of metanephrine produced were assessed for statistical significance by unpaired *t*-test. Differences among the meninges (dura, pia, and pia-arachnoid) in the amount of metanephrine produced were assessed by one-way analysis of variance, and Student-Newman-Keuls test was used for post-hoc testing. A P value of <0.05 was considered statistically significant. All values are reported as mean ± SE.

Results

All meningeal samples from both species demonstrated metabolism of epinephrine to metanephrine (table 1). Intact monkey meningeal specimens (dura-arachnoid-pia mater) generated metanephrine at the rate of 0.47 ± 0.19 ng · min^{-1} · cm^{-2}, and intact pig meninges generated metanephrine at the rate of 0.23 ± 0.04 ng · min^{-1} · cm^{-2} ($P > 0.05$). There was a significant difference in the amount of metanephrine produced by the different pig meningeal tissues (table 2). The pia-arachnoid meninx produced metanephrine at a greater rate per milligram of tissue (4.48 ± 1.04 ng · min^{-1} · mg^{-1}) than did the pia mater (1.3 ± 0.15 ng · min^{-1} · mg^{-1}) or dura mater alone (1.82 ± 0.23 ng · min^{-1} · mg^{-1}). There was no difference between the dura mater and the pia mater in the rate at which they generated metanephrine on a per-milligram basis. Metanephrine production per square centimeter of tissue was significantly greater in the pia-arachnoid (28.42 ± 2.33 ng · min^{-1} · cm^{-2}) than in the dura (17.11 ± 1.49 ng · min^{-1} · cm^{-2}), which in turn was significantly greater than metanephrine production by the pia mater alone (5.03 ± 0.84 ng · min^{-1} · cm^{-2}).

Control studies confirmed that epinephrine did not undergo spontaneous oxidation in this experimental preparation, thus metanephrine production was not limited by nonenzymatic degradation of epinephrine substrate. In addition, epinephrine metabolites were not produced in the absence of exogenous epinephrine, indicating that the metanephrine metabolites detected resulted from metabolism of the exogenously administered epinephrine.

Table 1. Metanephrine Production by Monkey and Pig Spinal Meninges

Metanephrine Production	Monkey	Pig	*P* Value
ng · ml^{-1} mock CSF	376.5 ± 151.9	182.6 ± 33.5	NS
ng · min^{-1} · cm^{-2} of tissue	0.47 ± 0.19	0.23 ± 0.04	NS

CSF = cerebrospinal fluid; NS = not significant.

Table 2. Amount of Metanephrine Produced by Different Meninges of the Pig

	Dura	Pia	Pia-arachnoid
ng · min^{-1} · mg^{-1} of tissue	1.82 ± 0.53	1.3 ± 0.33	4.48 ± 1.04†
ng · min^{-1} · cm^{-2} of tissue	17.11 ± 1.49	5.03 ± 0.84*	28.42 ± 2.33†

* $P < 0.05$ (pia *vs.* dura).

† $P < 0.05$ (pia-arachnoid *vs.* dura and pia).

Discussion

These data demonstrate that the spinal meninges of both pigs and monkeys are able to metabolize epinephrine to metanephrine. This reaction is catalyzed by COMT, which facilitates the transfer of a methyl group from S-adenosyl-methionine to a variety of catechol compounds.[17] Thus, these data indicate the functional presence of COMT in the spinal meninges of both pigs and monkeys. The physiologic role of COMT in the spinal meninges is not yet clear, but in the cranial meninges, it is thought to play an important role in eliminating endogenous catechol neurotransmitters from the CSF, thereby preventing their reentry into the brain.[13,18] A similar role for the spinal meninges would seem likely. Given that the spinal meninges of these two species are able to metabolize epinephrine, we speculate that human spinal meninges also contain COMT and are able to metabolize epinephrine.

Because the arachnoid mater is thin and delicate, we were unable to separate it intact from the pia mater to weigh it directly. Thus, we cannot precisely calculate the rate of metanephrine production per milligram of arachnoid tissue. However, the fact that the pia-arach-

noid mater of the pig produced significantly more metanephrine than either the pia alone or the dura alone suggests that the arachnoid mater is the principal site of epinephrine metabolism.

The important clinical question is whether epinephrine metabolism by spinal meningeal COMT prevents epidurally administered epinephrine from reaching analgesic sites in the spinal cord dorsal horn. This *in vitro* study cannot answer this question directly, but the results seem to clearly indicate that metabolism of epinephrine as it diffuses through the spinal meninges will reduce the spinal bioavailability of epidurally administered epinephrine. In addition, this study suggests that intrathecally administered epinephrine may be eliminated, in part, by metabolism within the spinal meninges.

These data also suggest that the conventional view of the spinal meninges may need revision. The spinal meninges have traditionally been thought of as simple anatomic barriers, with the thicker dura mater accounting for most of the resistance to drug diffusion.[19–21] However, Bernards and Hill have demonstrated that the cellular arachnoid mater is the major meningeal diffusion barrier between the epidural space and the spinal cord.[22] The current study extends this earlier work by demonstrating that the arachnoid mater may be an important metabolic barrier in addition to a physical barrier. Thus, just as the blood-brain barrier provides physical and metabolic protection of the central nervous system, the spinal meninges may protect the underlying spinal cord from exogenous or endogenous molecules.

Additional studies are necessary to further our understanding of the basic physiology of the spinal meninges. If we are to intelligently use the epidural space as a drug delivery route for spinally active drugs, it is essential that we understand the role of the meninges in limiting the spinal bioavailability of epidurally administered drugs.

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