Vasopressin contributes to dynorphin modulation of hypoxic cerebrovasodilation

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Venteicher, Andrew, and William M. Armstead. Vasopressin contributes to dynorphin modulation of hypoxic cerebrovasodilation. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H2072–H2079, 1998.—Because pial artery dilation during a 20- or 40-min hypoxic exposure was less than that observed during a 5- or 10-min exposure, stimulus duration determines the vascular response to hypoxia. Dynorphin (Dyn) modulates hypoxic pial dilation and contributes to decremented dilation during longer hypoxic exposures. This study was designed to determine whether vasopressin (VP) contributes to Dyn modulation of hypoxic pial dilation in newborn pigs equipped with a closed cranial window. Moderate (M) and severe (S) hypoxia (arterial PaO₂ = 35 and 25 mmHg, respectively) had no effect on cerebrospinal fluid VP during a 5-min exposure but increased its concentration during longer exposure periods. The VP antagonist [D-erythropophin]-amidinophenyl]-D-Me-Tyr²,Arg⁶,-vasopressin (MEAVP) had no effect on pial dilation during the 5-min exposure but potentiated the 20- and 40-min M and S hypoxic exposure dilations: 21 ± 2 vs. 29 ± 3% and 23 ± 2 vs. 33 ± 2% for 20- and 40-min S hypoxic dilation before and after MEAVP. Topical VP during 5 min of hypoxia elicited dilation that was reversed to vasoconstriction during 20 min of S hypoxia. Similarly, during 5 min of hypoxia, VP elicited dilation that was reversed to vasoconstriction during longer hypoxic periods. MEAVP blunted this Dyn-induced vasoconstriction. These data show that VP modulates hypoxic pial dilation in a stimulus duration-dependent manner and that VP contributes to the reversal of Dyn from a dilator to a constrictor during prolonged hypoxia. Finally, these data suggest that VP contributes to Dyn modulation of hypoxic cerebrovasodilation.

VASOPRESSIN CONTRIBUTES to the regulation of cerebral hemodynamics. Previous studies have shown vasopressin to produce cerebral artery vasoconstriction in the cat, goat, and human (11, 18, 22), dilation in the cat and dog (14), or no effect in the rat (16). Although there may be species and/or regional vascular differences, a possible explanation for such divergent observations could be that vasopressin’s actions are tone dependent. For example, in the piglet, vasopressin elicits dilation during resting tone and vasoconstriction when cerebrovascular tone is decreased (6).

Several mechanisms have been proposed to account for hypoxia-induced cerebrovasodilation. These possibilities include opioids, vasopressin, adenosine, prostaglandins, and nitric oxide (NO) (9, 10, 13, 21). For example, it was observed that hypoxia increases plasma methionine enkephalin in fetal sheep (20) and plasma β-endorphin in human newborns at delivery (19, 27) and in infants with hypoxic ischemic encephalopathy with ongoing hypoxia (24). In the newborn pig, hypoxia for 10 min was associated with elevated cortical periventricular cerebrospinal fluid (CSF) levels of the opioids methionine enkephalin, leucine enkephalin, and dynorphin (1, 2), which are μ-, δ-, and κ-opioid agonists, respectively. Because μ- and δ-receptor antagonists attenuated, whereas a κ-antagonist potentiated, hypoxic pial artery dilation, these data indicate that methionine enkephalin and leucine enkephalin contribute, whereas dynorphin opposes, hypoxic pial dilation (1, 2). However, recent data indicate that such modulation of hypoxic pial dilation by opioids is dependent on the duration of hypoxia. For example, if hypoxic methionine enkephalin concentration was unchanged during a 5-min exposure and increased during a 10-min exposure but decreased during 20- and 40-min hypoxic exposures (4). A μ-opioid antagonist had no influence on dilation during the 5-min exposure and decremented the 10- and 20-min exposures but had no effect on the 40-min exposure to hypoxic dilation. In contrast, if dynorphin concentration was elevated for all except the 5-min hypoxic exposure, and a κ-antagonist potentiated 10-, 20-, and 40-min exposure dilation (4). Interestingly, hypoxic pial dilation was diminished during longer exposure periods (4). These data, therefore, indicated that such decremented hypoxic pial dilation during longer exposure periods resulted from decreased release of methionine enkephalin and accentuated release of dynorphin. Because dynorphin also is a tone-dependent agent like vasopressin (6) and hypoxia decreases cerebral tone, these data suggest that dynorphin reverses from a dilator to a vasoconstrictor during hypoxia. In the piglet, 10 min of hypoxia also increased CSF vasopressin, whereas a vasopressin antagonist attenuated hypoxic pial artery dilation, indicating that vasopressin contributes to the vascular response during this stimulus (23). In unrelated studies it had been observed earlier that vasopressin contributed to the reversal of dynorphin from a dilator to a constrictor during decreased tone conditions (5, 8). However, the role of vasopressin in the vascular response during longer hypoxic exposures is uncertain. Equally uncertain is the ability of vasopressin to contribute to the dynorphin-induced modulation of hypoxic pial artery dilation.

Therefore, the present study was designed to determine whether vasopressin contributes to dynorphin modulation of hypoxic pial artery dilation by 1) charac-
terizing the contribution of endogenous vasopressin to hypoxic pial dilation as a function of exposure duration, 2) determining the vascular response to exogenous vasopressin during hypoxia as a function of exposure duration, and 3) determining the contribution of vasopressin to the vascular response of exogenous dynorphin during hypoxia as a function of hypoxic exposure duration.

METHODS

All experiments have been approved by the Institutional Animal Care and Use Committee. Pigs (1–5 days old) of either gender were anesthetized with ketamine hydrochloride-acepromazine (33 mg/kg im). Anesthesia was maintained with α-chloralose (30–50 mg/kg initially, supplemented with 5 mg/kg iv). A catheter was inserted into the femoral artery to record blood pressure and to sample for blood gases and pH. Another catheter was placed in a femoral vein for injection of drugs. The trachea was cannulated, and the animals were ventilated with room air. The body temperature was maintained at 37–38°C with a heating pad.

For insertion of the cranial window, the scalp was removed and an opening was made in the skull over the parietal cortex. The dura was cut and retracted over the cut bone edge. The cranial window was placed in the hole and cemented in place with dental acrylic. The space under the window was filled with artificial CSF of the following composition (in mM): 3.0 KCl, 1.5 MgCl2, 1.5 CaCl2, 132 NaCl, 6.6 urea, 3.7 dextrose, and 24.6 NaHCO3 (pH 7.30–7.36, P CO2 42–49 mmHg, and PO2 40–50 mmHg).

Pial arterioles were observed with a dissecting microscope, a television camera mounted on the microscope, and a video monitor. Vascular diameter was measured with a video microcaler.

Protocol. Animals were divided into 16 groups: 1) 5 and 10 min of moderate and severe hypoxia time control (n = 5), 2) 20 min of moderate and severe hypoxia time control (n = 5), 3) 40 min of moderate and severe hypoxia time control (n = 5), 4) 5 and 10 min of moderate and severe hypoxia before and after [α-Me-Tyr2] vasopressin (MEAVP, n = 8), 5) 20 min of moderate and severe hypoxia before and after MEAVP (n = 8), 6) 40 min of moderate and severe hypoxia before and after MEAVP (n = 8), 7) lysine vasopressin (LVP) after 5 and 10 min of moderate and severe hypoxia (n = 8), 8) LVP after 20 min of moderate and severe hypoxia (n = 8), 9) LVP after 40 min of moderate and severe hypoxia (n = 8), 10) dynorphin after 5 and 10 min of moderate and severe hypoxia (n = 8), 11) dynorphin after 20 min of moderate and severe hypoxia (n = 8), 12) dynorphin after 40 min of moderate and severe hypoxia (n = 8), 13) dynorphin after 5 and 10 min of moderate and severe hypoxia in MEAVP-pretreated animals (n = 8), 14) dynorphin after 20 min of moderate and severe hypoxia in MEAVP-pretreated animals (n = 8), 15) dynorphin after 40 min of severe hypoxia in MEAVP-pretreated animals (n = 8), and 16) LVP and dynorphin time controls (n = 5). Time control experiments were designed so that responses were obtained initially (time 1 in Fig. 1), then again 30 min later (time 2 in Fig. 1). Severity and duration of hypoxia were randomized within groups.

Hypoxia (5, 10, 20, and 40 min) was produced by decreasing the inspired O2 sufficiently to reduce and maintain arterial PO2 (PaO2) at 35 ± 5 and 25 ± 3 mmHg (moderate and severe hypoxia, respectively) while maintaining constant arterial PCO2 in the normocapnic range (33 ± 3 mmHg). Changes in pial artery diameter (120–160 and 50–70 µm for small artery and arteriole, respectively) were measured every minute during the last 5 min of each hypoxic exposure period. Two sizes of pial arteries were investigated to determine whether regional vascular differences with respect to the modulation of hypoxic dilation by vasopressin could be observed. A sample of blood confirming the hypoxia was taken 3 min after the hypoxia began. Once the blood chemistry data confirmed that the desired level of hypoxia had been achieved, dilator responses were recorded. In animals exposed to hypoxia for longer periods, dilator responses were also recorded during the initial 10 min of exposure to confirm that these animals had responded appropriately to the stimulus. Therefore, differences observed at 20 or 40 min of hypoxic exposure would not be due to an initially aberrant response. Responses to hypoxia were obtained before and after MEAVP (5 µg/kg iv; Sigma Chemical, St. Louis, MO). This dose of MEAVP has previously been shown to inhibit responses to topically applied vasopressin (6). For hypoxia experiments in the presence of MEAVP, this inhibitor was systemically administered 30 min before induction of hypoxia, and the effects of the inhibitor on hypoxia-induced pial arterial dilation were observed for the succeeding variable (5-, 10-, 20-, or 40-min exposure period).
Cortical periarachnoid CSF was collected during the last 10 min of each hypoxic exposure period and therefore represents the amount of vasopressin released after a given stimulus period. Needles incorporated into the side of the cranial window allowed for the injection of CSF under the window and the runoff of excess CSF. For sample collection, 300 µl of CSF were collected from under the cranial window, which has a total volume of 500 µl, thereby minimizing dilution of the sample. The CSF (300 µl) was collected by slowly infusing artificial CSF into one side of the window and allowing the CSF under the window to drip freely into a collection tube on the opposite side.

To investigate the effects of hypoxia on responses to vasopressin and dynorphin, LVP (40, 400, and 4,000 pg/ml; Sigma Chemical) and dynorphin (10^-10, 10^-8, and 10^-6 M; Sigma Chemical) were topically applied before and during moderate and severe hypoxia (5, 10, 20, and 40 min). The concentrations of vasopressin investigated in this study were chosen to reflect the concentrations in CSF at rest (40 pg/ml), long hypoxic stimulation (400 pg/ml), or pharmacological (4,000 pg/ml) conditions. For these experiments, these agents were applied during hypoxia but at the end of the respective time period. Data for dynorphin and vasopressin responses were calculated by obtaining the percent change in baseline diameter from that obtained during hypoxia alone for each period of hypoxic exposure. LVP is the form of vasopressin in the pig. Appropriate aliquots of the vehicle for all agents (0.9% saline) were added to the CSF infused under the window. This CSF vehicle had no effect on pial artery diameter.

Vasopressin analysis. CSF samples were immediately frozen and stored at -20°C. RIA kits for vasopressin are commercially available (Linstar). The RIA uses simultaneous addition of sample, rabbit anti-vasopressin antibody, and the 125I-derivative of vasopressin. The antibody used in this RIA did not cross-react significantly with oxytocin or vasotocin (<0.5% cross-reactivity). After an overnight incubation at 4°C, free vasopressin was separated from vasopressin bound to the antibody by the addition of a precipitating complex consisting of guinea pig serum precipitated with goat anti-guinea pig serum and polyethylene glycol. After centrifugation at 760 g for 20 min, the supernatant was decanted, and the pellet was counted with a gamma scintillation counter. All samples and standards were assayed in duplicate. Data are calculated as %B/B0 vs. concentration, where B/B0 is [(average cpm of sample − average cpm of nonspecific binding tube) (average cpm of total binding tube − average cpm of nonspecific binding tube)] × 100.

Statistical analysis. Pial artery diameter, systemic arterial pressure, and vasopressin values were analyzed using repeated-measures analysis or t-test where appropriate. If the F value was significant, Fisher's test was performed on all data analyzed by repeated measures. P < 0.05 was considered significant in all statistical tests. The n values reflect data for one vessel in each animal. Values are means ± SE of absolute values or as percent change from control values. Data presented as percent change were also compared by nonparametric means with the Wilcoxon signed rank test.

RESULTS

Contribution of vasopressin to hypoxic pial artery dilation as a function of stimulus duration. Moderate and severe hypoxia (PaO2 ~ 35 and 25 mmHg, respectively) elicited reproducible pial small artery (120–160 µm) and arteriole (50–70 µm) dilation during 5-, 10-, 20-, and 40-min exposure periods (Fig. 1). Although 5 min of hypoxia produced dilation of magnitude quite similar to that observed during a 10-min exposure period (Fig. 1), the dilation seen during 20 and 40 min was decreased from that observed during 5 or 10 min of hypoxic exposure (Fig. 2). Moderate and severe hypoxia had no effect on cortical periarachnoid CSF vasopressin concentration during a 5-min stimulus period (Fig. 3). During 10-, 20-, and 40-min exposure periods, however, vasopressin concentration increased steadily with the lengthening of the exposure period (Fig. 3). The vasopressin antagonist MEAVP (5 µg/kg iv) had no influence on dilation during the 5-min exposure and decremented the 10-min moderate and severe and the 20-min moderate hypoxic exposure but potentiated the 20-min severe and the 40-min moderate and severe hypoxic exposure dilations (Fig. 2). The potentiation by MEAVP during 20 min of severe and 40 min of moderate or severe hypoxia was sufficiently great as to make those responses, typically diminished because of the effects of longer exposure to hypoxia, no different from the response observed during shorter (5 or 10 min).
min of moderate hypoxic exposure (Fig. 5). Dynorphin dilution during normoxia (0 min of hypoxia in Fig. 5, C and D) was potentiated by MEAVP compared with corresponding normoxic values in the absence of MEAVP (Fig. 5, A and B). Although MEAVP had no effect on dynorphin-induced dilution during 5 min of moderate hypoxia (Fig. 5), such responses during 5 min of hypoxia were smaller than corresponding normoxic values because of the aforementioned MEAVP potentiation of dynorphin dilution during normoxia (Fig. 5, C and D). MEAVP modestly accentuated the reversal of dynorphin from a dilator to a constrictor during 10 min of moderate hypoxia but blunted such reversal during 20 and 40 min of moderate hypoxia (Fig. 5, C and D). MEAVP had similar effects on dynorphin vascular responses during severe hypoxia (data not shown).

Blood chemistry and mean arterial blood pressure. Blood chemistry and mean arterial blood pressure values were obtained at the beginning and end of all experiments as well as during hypoxia. Hypoxia decreased PaO2, as expected (35 ± 3, 25 ± 3, and 93 ± 4 mmHg for moderate hypoxia, severe hypoxia, and normoxia, respectively) whereas the pH, arterial Pco2, and mean arterial blood pressure values were unchanged (7.44 ± 0.01, 33 ± 2, and 68 ± 4 mmHg vs. 7.43 ± 0.01, 34 ± 3, and 66 ± 5 mmHg, respectively).

DISCUSSION

Results of the present study show that although 5 min of hypoxia produced pial artery dilatation of magnitude quite similar to that observed during 10 min of exposure, the dilatation seen during 20 and 40 min was decreased from that observed during 5 or 10 min of hypoxic exposure. These results indicate that the duration of the stimulus determines the nature of the vascular response to hypoxia, consistent with a recent study (4). New data in the present study show that moderate and severe hypoxia had no effect on cortical periarachnoid CSF vasopressin concentration during a 5-min stimulus period. Concomitantly, the vasopressin antagonist MEAVP had no effect on pial dilatation during a 5-min hypoxic stimulus. Taken together, the biochemical data support and corroborate the pharmacological data and indicate that vasopressin does not contribute to hypoxic pial dilatation when the stimulus period is 5 min.

In contrast, during a 10-min hypoxic stimulation, vasopressin CSF concentration was increased, consistent with previous observations (23). New data in the present study show that CSF vasopressin concentration continued to increase during 20- and 40-min exposure periods. MEAVP decremented the 10-min moderate and severe and the 20-min moderate hypoxic exposure but potentiated the 20-min severe and the 40-min moderate and severe hypoxic exposure dilations. Vascular responses during 20 and 40 min of moderate or severe hypoxia were typically diminished because of the effects of longer hypoxic exposure. However, the potentiation by MEAVP made such responses different from the response observed during shorter (5- or 10-min) exposures. Thus vasopressin contributes
to 10-min moderate and severe and 20-min moderate hypoxic pial dilation. The latter data suggested, however, that vasopressin contributes to decremented hypoxic pial artery dilation during longer exposure periods. Taken together, these data indicate that the role of vasopressin in hypoxic pial artery dilation is stimulus duration dependent. Systemically administered MEAVP had previously been observed to block vascular responses to topical vasopressin (6). MEAVP by itself, however, had no effect on pial artery diameter, indicating that there was little tonic vasopressin contribution to resting vascular tone.

Because vasopressin is a tone-dependent agent (6), observations that MEAVP restored decremented hypoxic pial dilation during longer exposure periods toward values obtained using shorter exposure periods suggest that vasopressin reversed from a dilator to a vasoconstrictor during such longer periods. New data in this study, in fact, show this to be the case. For example, exogenously administered vasopressin-induced dilation was unchanged during 5- and 10-min hypoxic exposures. However, vasopressin-induced pial artery dilation was decremented during 20 min of moderate hypoxia. During 20 min of severe hypoxia and 40 min of moderate or severe hypoxia, vasopressin-induced dilation was reversed to vasoconstriction. These data indicate that the duration of hypoxia can influence the vascular response to vasopressin.

Similar to vasopressin, the observation that a dynorphin antagonist partially restored decremented hypoxic pial dilation during long exposure periods (4) suggested that dynorphin also reversed from a dilator to a vasoconstrictor during hypoxia. Additional data in the present study show that dynorphin-induced dilation was unchanged during a 5-min hypoxic exposure but was reversed to vasoconstriction during 10-, 20-, and 40-min hypoxic exposures. In previous studies, vasopressin attenuated dynorphin-induced dilation during resting cerebrovascular tone but contributed to the reversal of dynorphin from a dilator to a vasoconstrictor during normoxic decreased cerebrovascular tone conditions (5, 8). At that time, it had been speculated that dynorphin-induced dilation resulted in a decreased cerebrovascular tone that reversed the tone-dependent agent vasopressin from a dilator to a constric- tor to oppose dynorphin dilation during normoxia. Such results were confirmed in the present study. New data in this study show that although MEAVP had no effect on dynorphin-induced dilation during the 5-min hypoxic exposure period, it modestly accentuated the rever-
sal of dynorphin from a dilator to a constrictor during 10-min moderate and severe hypoxic exposure periods. These data are consistent with the observations that vasopressin is a vasodilator during a 5- or 10-min hypoxic exposure, thereby opposing dynorphin-induced vasoconstriction that occurs during a 10-min hypoxic exposure. In contrast, MEAVP blunted the reversal of dynorphin from a dilator to a constrictor during 20- and 40-min hypoxic exposure periods. These data are consistent with the observation that vasopressin is a vasoconstrictor during such exposure periods and would therefore contribute to the reversal of dynorphin from a dilator to a constrictor. These data then suggest that vasopressin contributes to dynorphin modulation of hypoxic cerebrovasodilation. Table 2 summarizes the effects of dynorphin and vasopressin on pial artery diameter as well as the interactions between these two agents as a function of hypoxic exposure duration. Table 2 shows that although vasopressin has no effect on dynorphin dilation during 5 min of hypoxia, it opposes dynorphin constriction during 10 min of hypoxic exposure and contributes to such vasoconstriction during 20- and 40-min hypoxic exposures.

The choice of 10 min as the duration of hypoxia in previous studies was arbitrary. Clinically, episodes of acute hypoxia are variable in duration and often last for

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**Table 2. Influence of dynorphin, vasopressin, and vasopressin-dynorphin interaction on pial artery diameter as a function of hypoxic duration**

<table>
<thead>
<tr>
<th>Hypoxia</th>
<th>5 min</th>
<th>10 min</th>
<th>20 min</th>
<th>40 min</th>
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<tbody>
<tr>
<td></td>
<td>M</td>
<td>S</td>
<td>M</td>
<td>S</td>
</tr>
<tr>
<td>Dynorphin</td>
<td>D</td>
<td>D</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>Vasopressin</td>
<td>D</td>
<td>D</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>Vasopressin influence on dynorphin</td>
<td>No effect</td>
<td>Opposes C</td>
<td>Less D</td>
<td>C</td>
</tr>
</tbody>
</table>

M, moderate; S, severe; D, vasodilation; C, vasoconstriction.
>10 min. Recent data from Leffler et al. (17) in the newborn pig show that NO and activation of ATP-dependent K⁺ channels do not contribute to pial artery dilation during 5 min of hypoxia. Because previous data from this laboratory show that NO and activation of such K⁺ channels contribute to pial artery dilation during 10 min of hypoxia in the piglet (1, 25, 28), these studies together suggest that the relative importance of other mechanisms involved in hypoxic pial dilation changes as a function of the duration of the stimulus.

The origin of the vasopressin detected in CSF cannot be determined from the present experiments. The presence of vasopressin-immunoreactive nerve fibers has been demonstrated in guinea pig pial arteries (12). Furthermore, it has been reported that vascular tissues derived from pial vessel stores for vasopressin or from nerves associated with those vessels. Because MEA VP blocked vasodilation to topical vasopressin (6) and has been reported to be a V₁ antagonist (15), this CSF vasopressin appears to predominantly interact with the V₁ receptor in the piglet cerebral circulation. Such vasodilation is dependent on the release of NO (3, 14). Interestingly, the constrictor component for vasopressin during reduced-tone conditions is also dependent on activation of the same V₁ receptor (6). MEA VP, however, is specific for antagonism of vasopressinergic receptors in the piglet cerebral circulation, since it has been previously observed that responses to norepinephrine, the thromboxane mimic U-46619, and isoproterenol were unchanged by this antagonist (6).

In conclusion, results of the present study show that vasopressin modulates hypoxic pial artery dilation in a duration-dependent manner. These data also show that vasopressin contributes to the reversal of dynorphin from a dilator to a vasoconstrictor during prolonged hypoxia. Finally, these data suggest that vasopressin contributes to dynorphin modulation of hypoxic cerebrovasodilation.

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