MINIREVIEW

Role of Opioids in the Physiologic and Pathophysiologic Control of the Cerebral Circulation[1] (44089)

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The cerebral circulation is regulated by chemical stimuli, metabolic factors, perfusion pressure, and nerves. A previous review focused on local humoral control of a paracine-autocrine nature and on selected novel (vasopressinergic, opioid) and classical (sympathetic, cholinergic) neural-humoral stimuli for the control of the cerebral circulation (1). The present review will serve as a follow-up to the previous article and will focus on the role of opioids in the physiologic and pathophysiologic control of the cerebral circulation.

Opioids and the Physiologic Control of the Cerebral Circulation

General Observations. Opioids contribute to the regulation of cerebral hemodynamics. Opioid receptor binding has been demonstrated on cerebral microvessels (2). Enkephalin and dynorphin immunoreactivity, indicative of innervation, has been shown in large cerebral arteries of the pig and guinea pig, respectively (3, 4). Furthermore, opioids have been detected in cerebrospinal fluid (CSF) (5) and CSF opioid concentrations are in the vasoactive range under control conditions in the newborn pig (6, 7).

Investigations into the ability of opioids to influence the cerebral circulation have resulted in conflicting data. For example, enkephalins have been observed to produce modest dilation in isolated feline middle cerebral arteries, minimal dilation in feline pial arteries at very high doses, and no effect on canine basilar arteries (8–10). Synthetic κ-opioids agonists, such as ethylketocyclazocine, and bremazocine, additionally, have been observed to elicit dilation of canine basilar and middle cerebral arteries (11). Responses after activation of cerebrovascular opioid receptors have been less well characterized in *in vivo* compared with *in vitro* studies. Topical methionine enkephalin, for example, has been shown to decrease feline cortical blood flow (12), while others have observed this opioid to elicit biphasic responses, first increasing then decreasing cerebral blood flow (13). Alternatively, systemically administered (D Met^2, Pro^5) enkephalinamide, a synthetic enkephalin analog, dilated feline pial vessels (14), while the intracisternal administration of another analog, D-Ala-Leu-enkephalin, increased cerebral blood flow (15). Moreover, others have observed that anesthetic agents can influence the cerebrovascular activity of opioids. Depending on the absence or presence, as well as the level, of anesthesia, opioids have been observed to increase, decrease, or have no effect on cerebral blood flow (16, 17). Therefore, the baseline metabolic rate could influence the cerebrovascular activity of opioids.

In contrast to the somewhat inconsistent results from the above studies in adult animals, topically applied opioids have prominent effects on newborn pig pial arteries *in vivo*. Although synthetic analog possessing higher affinity for opioid receptor subtypes is available, these initial studies were designed to evaluate the cerebrovascular activity of naturally occurring opioids. These results indicate that μ (methionine enkephalin) (18) and δ (leucine enkephalin) (18) receptor activa-

[1] This manuscript is an update of a previously published minireview entitled. "Neurohumoral Regulation of the Cerebral Circulation" (Proc Soc Exp Biol Med **199**:149–157, 1992).

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P.S.E.B.M. 1997, Vol. 214

0037-9727/97/2143-0210$10.50/0

tion elicit dilation whereas ε (β endorphin) (18) receptor activation produces pial vasoconstriction (6, 7). κ (Dynorphin) (18) receptor activation produces tone-dependent responses (dilation during resting tone, constriction when cerebrovascular tone is decreased) (6, 7). Using selective synthetic opioid receptor analogs and antagonists as probes in subsequent studies, it was later confirmed that these physiological responses were correlated with activation of the above opioid receptor subtypes. For example, the μ agonist, DAMGO, and methionine enkephalin elicited pial dilation that was blocked by the μ receptor antagonist, β funaltrexamine, but unchanged by BNTX, naltrindole, or norbinaltorphimine, δ_1, δ_2, and κ opioid receptor antagonists respectively (19–24). Similar cross-selectivity pharmacologic experiments corroborate physiologic data in earlier studies with the endogenous δ and κ agonists, leucine enkephalin and dynorphin (19, 20). Additionally, leucine enkephalin interacts with both δ_1 and δ_2 receptors in an equivalent manner; approximately 50% of the vascular response was removed with either BNTX or naltrindole administration (20). Moreover, tone-dependent responses were also observed for the synthetic κ agonist, U50,488H (25, 26), indicating that such a vascular profile is dependent on κ opioid receptor activation. Although the mechanisms for tone-dependent responses are not known, alterations in the physical state of cell membranes that occur during dilation and constriction may play a role in the tone dependent nature of vascular responses. It is speculated that such membrane changes could result in altered receptor-effector coupling mechanisms and associated upregulation/downregulation of intracellular signaling mechanisms.

Finally, it should be cautioned that most of the above discussion focuses on observations made in the newborn pig. The reasons for differences between these data and previously published studies in the adult with respect to the nature of opioid cerebrovascular responses are uncertain but could be due to developmental differences in opioid receptor density and/or signal transduction.

Mechanisms Involved in Opioid Cerebrovascular Activity. The mechanisms for opioid-induced vascular activity has been of considerable interest. Opioid-induced vascular effects could be caused directly by opioids acting on vascular receptors, or indirectly as a consequence of an alteration in metabolism. However, it has been observed that opioid-induced vascular effects do not result from secondary changes in cerebral metabolic utilization of glucose in the newborn pig (27). On the other hand, nitric oxide (NO) and the production of cGMP contribute to pial artery vasodilation to the endogenous opioids methionine enkephalin, leucine enkephalin, and dynorphin (28). NO also contributes to pial dilation elicited by the synthetic analogs DAMGO, DPDPE, deltorphin, and U50,488H, μ, δ_1, δ_2, and κ receptor agonists, respectively (28–31). Data showing stereo selective reversal of the vasodilatory attenuation by the NO synthase inhibitor L-NNA, with L- versus D-arginine in conjunction with appropriate changes in CSF cGMP levels further strengthens the interpretation that NO contributes to opioid-induced pial vasodilation (28). Because such changes in vascular and biochemical parameters are blocked by naloxone (7, 28), these effects are opioid receptor mediated. Taken together, these data indicate that NO contributes to pial dilation induced by activation of μ, δ_1, δ_2, and κ opioid receptors, κ opioid-induced dilation being somewhat more dependent on the release of NO than other opioid receptor subtypes (28).

Additionally, it has been observed that activation of ATP-sensitive $K^+(K_{ATP})$ channels contribute to pial artery dilation produced by methionine enkephalin, leucine enkephalin, dynorphin, DAMGO, and DPDPE (32). In contrast, pial dilation in response to deltorphin and U50,488H was unchanged by the K_{ATP} channel antagonist, glibenclamide (32). Because dynorphin was dependent and U50,488H independent of K_{ATP}-channel activation, these data support the idea that there are several κ isoreceptors (33), each coupled to different second messenger systems. While the above studies were the first to investigate the role of K_{ATP}-channel activation in the vascular activity of opioids, other types of studies have noted an interaction of opioid receptors with K_{ATP} channels. For example, based on antinociception studies, it has been suggested that μ and δ_1 agonists activate K_{ATP} channels, whereas δ_2 agonists do not (34, 35). Additional electrophysiologic studies support the interaction between μ opioid receptors and K_{ATP} channels, while also indicating that there may not be an interaction between κ opioid receptors and this ion channel (36). Finally, there could be a link between the observations that both NO and activation of K_{ATP} channels contribute to methionine enkephalin, leucine enkephalin, and dynorphin pial dilation. For example, it has recently been observed that activation of K_{ATP} channels contributes to cGMP-mediated pial artery dilation (37). Therefore, these data suggest that the above endogenous opioids elicit pial dilation *via* the sequential release of NO and cGMP, which in turn activates the K_{ATP} channel.

On the other hand, prostaglandins have also been observed to contribute to opioid-induced vasodilation. This idea is supported by the results of several studies showing that morphine and methionine-enkephalin are able to increase prostaglandin production in brain homogenates (38, 39). Using cyclooxygenase inhibitors as probes, it has also been observed that prostaglandins mediate methionine-enkephalin–induced depressor responses in the cat (40). Further, methionine-enkephalin, leucine-enkephalin, and dynorphin-induced pial artery dilation in the piglet has been observed to be accompa-

nied by increased CSF prostaglandins and blunted by indomethacin (7).

Opioid-induced elevation of CSF cGMP concentration due to NO and CSF cAMP due to dilator prostaglandins may suggest an interaction between the two cyclic nucleotides in the elicitation of the vasodilator response. Thus, the increases in cortical cGMP observed during opioid receptor activation could be an indirect consequence of elevated cAMP as a result of cross-talk between cyclic nucleotide metabolizing systems at the level of their synthesis or degradation (41). For example, it has been suggested that an increase in cAMP levels would competitively prevent cGMP breakdown at the level of phosphodiestrase (41, 42). Furthermore, it has been observed that forskolin, a direct activator of adenylate cyclase, induced pial arterial dilation and increased CSF cGMP; the vascular and biochemical changes being attenuated by L-NNA (43). These data suggest an association between cAMP elevation and the consequent activation of NO synthase. It is presently uncertain if this possibility could account for the dual involvement of NO and prostaglandins in opioid-induced pial artery vasodilation.

In addition to contributing to opioid-induced pial artery dilation, prostaglandins can also modulate opioid-induced pial constriction. For example, indomethacin potentiates β-endorphin–induced constriction and dynorphin-induced constriction during hypotension (7). Therefore, prostaglandins attenuate β-endorphin–induced constriction and the constriction caused by dynorphin in hypotensive piglets.

Alternatively, opioids could contribute to the regulation of cerebral hemodynamics through interactions with other vasoactive systems. For example, opioids have been observed to be localized with vasopressin in the posterior pituitary and could, therefore, influence vasopressin secretion (44). Previously, it has been shown that opioids can both stimulate and inhibit plasma vasopressin release (45, 46). Additionally, it has been shown that dynorphin increases plasma vasopressin concentration and that a V_1 receptor antagonist blocked the pressor response to dynorphin in the fetal lamb (47). Recently, it has been observed that vasopressin modulates opioid cerebrovascular responses in the newborn pig (26, 48). For example, dynorphin-induced dilation, β-endorphin–induced constriction, and dynorphin-induced constriction during hypotension were all associated with increased periarachnoid cortical CSF vasopressin concentration (26). Furthermore, a V_1 receptor antagonist potentiated dynorphin-induced dilation, but attenuated β-endorphin–induced constriction and the constriction produced by dynorphin during hypotension (48). In contrast, responses to methionine enkephalin and leucine enkephalin were not associated with a change in CSF vasopressin concentration and the dilator responses were unchanged by a V_1 receptor antagonist (26, 48). Therefore, vasopressin appears to attenuate dynorphin-induced dilation, but to contribute to β-endorphin–induced constriction and constriction to dynorphin during hypotension through the activation of V_1 receptors. Since PGI_2 increases CSF vasopressin and two different cyclooxygenase inhibitors (indomethacin and aspirin) blunted dynorphin and β-endorphin–induced vasopressin release, the mechanism whereby opioids produce an increase in CSF vasopressin concentration is prostaglandin dependent in the piglet (49).

The origin of the vasopressin, however, cannot be determined from the above experiments. The presence of vasopressin-immunoreactive nerve fibers has been demonstrated in guinea pig pial arteries (50). Further, it has been reported that vascular tissue obtained from the aorta, vena cava, renal artery, and the mesenteric artery of both Sprague-Dawley and hypophysectomized rats contain immunoreactive stores of vasopressin (51). These data suggest that vascular stores of vasopressin could be of nonpituitary origin (51). Although the cellular site of origin is uncertain, the vasopressin detected in CSF, therefore, could be locally derived from pial vessel stores for vasopressin or from nerves associated with those vessels. Once released, this vasopressin could also affect the cerebral circulation, since similar vasopressin concentrations have been observed to produce dilation during normotension and constriction during hypotension (52).

Similar to studies involving the role of second messengers in opioid-induced dilation, the biochemical mechanisms involved in opioid-induced vasoconstriction have also received some attention. For example, it has been observed that dynorphin and synthetic κ opioid receptor agonists stimulate phosphoinositide turnover and elevate IP_3 concentration (53, 54). Results of a recent study extend these observations and indicate that activation of phospholipase C and protein kinase C are involved in dynorphin and β endorphin-induced pial artery vasoconstriction (55).

Additionally, there has also been some interest in the biochemical mechanisms involved in opioid release. For example, excitatory amino acids have been observed to release methionine enkephalin from slices of the rat striatum and globus pallidus (56), suggesting the involvement of nitric oxide in that release. Previous *in vitro* studies have alternately shown that isoproterenol or 8-Bromo cAMP causes the release of opioids from glial cells, adrenal chromaffin cells, and ventricular cardiac muscle cells, suggesting the involvement of cAMP in such release (57–59). More recently, it has been observed that sodium nitroprusside (SNP) and the stable cGMP analog 8-Bromo cGMP produce large increases in CSF methionine enkephalin and leucine enkephalin concentration (60). These biochemical changes and the pial artery dilation induced by these agents were blocked by the cGMP antagonist, Rp 8-Bromo

cGMPs. However, SNP and 8-Bromo cGMP had no effect on CSF dynorphin concentration. Additionally, Rp 8-Bromo cGMPs modestly decreased the resting CSF values for methionine enkephalin and leucine enkephalin (60).

In contrast, results of another study show that the cAMP analogs 8-Bromo cAMP and Sp 8-Bromo cAMPs produce marked increases in CSF dynorphin concentration and small increases in CSF methionine enkephalin and leucine enkephalin concentration (61). A cAMP antagonist, Rp 8-Bromo cAMPs, modestly decreased resting CSF values for methionine enkaphalin, leucine enkephalin, and dynorphin (61). Therefore, taken together these studies suggest that, while cGMP is more important relative to cAMP in elevating CSF methionine enkephalin and leucine enkephalin concentration, the converse is true for dynorphin. These studies also suggest that both cAMP and cGMP are involved in the tonic release of these opioids. Possible sources of these opioids include cortical vessels, nerves associated with these vessels, neurons, or glia. However, the origin of the opioids cannot be determined from the present experiments.

Although the concentration of CSF opioids under resting conditions is in the vasoactive range (61), naloxone has no effect on regional cerebral blood flow, cerebral vascular resistance, cerebral metabolic rate, or pial artery diameter in newborn pigs (7, 62). These data indicate that opioids have a more subtle influence on the cerebral circulation during resting physiologic conditions. However, as described below, opioids have a greater contribution to the regulation of cerebral hemodynamics during pathophysiologic conditions.

Opioids and the Pathophysiologic Control of the Cerebral Circulation

Role in the Cerebral Hemodynamic Effects of Hemorrhagic Hypotension. Several studies indicate that opioids interact with prostaglandins in autoregulatory vasodilation of the cerebral circulation during hemorrphagic hypotension. For example, in the piglet, pial artery dilation during hemorrhagic hypotension is associated with increased CSF prostaglandin concentration; indomethacin blocked these vascular and biochemical changes (63, 64). Indomethacin also caused a uniform decrease in cerebral blood flow, indicating that prostaglandins contribute to cerebral autoregulation during hemorrhage (65). As described in the above opioid physiology section, indomethacin potentiates dynorphin and β-endorphin–induced pial artery constriction, indicating that dilator prostaglandins inhibit constriction to these two opioids (7). Since naloxone blocked indomethacin-induced reductions in cerebral blood flow and increases in calculated cerebrovascular resistance (62), unopposed activation of constrictor opioid receptors could contribute to indomethacin-induced vasoconstriction during hemorrhagic hypotension. Moreover, CSF opioid concentrations increase during hemorrhagic hypotension (6). Additionally, naloxone attenuates the increase in CSF vasopressin concentration in response to hemorrhagic hypotension, indicating that opioids contribute to this pathophysiologic stimulus for vasopressin release (26). Interestingly, indomethacin also potentiates vasopressin-induced constriction (52), and a vasopressin receptor antagonist blocked indomethacin-induced constriction during hypotension, indicating that unopposed activation of vasopressingergic receptors also contributes to that constriction (66). Since dynorphin and β-endorphin elevate CSF vasopressin concentration (26) and the concentration of these two opioids are themselves elevated during hemorrhagic hypotension (6), opioids may also interact with prostaglandins in the control of the cerebral circulation during hemorrhagic hypotension *via* a secondary interaction with vasopressin. Therefore, several vasoactive systems could contribute to indomethacin-induced cerebral vasoconstriction during hemorrhagic hypotension.

Role in Hypoxia-Induced Pial Artery Dilation. Several mechanisms have been proposed to account for hypoxia-induced cerebral vasodilation. These possibilities include adenosine, prostaglandins, and endothelium-derived relaxant factor (67–69). Nitric oxide (NO), a putative endothelium-derived relaxant factor (70) has been suggested to be involved in hypoxia-induced cerebrovasodilation. For example, NO synthase inhibitors have been reported to attenuate the increase in cerebral blood flow elicited by hypoxia (71). On the other hand, NO synthase inhibitors have also been reported to have no effect on hypoxia-induced cerebrovasodilation (72, 73). These data indicate that there is a case, albeit controversial, for NO involvement, but only adult data are available. Because there is separate evidence available demonstrating the involvement of prostaglandins, vasopressin, and adenosine (67, 69, 74), these observations taken together indicate that other vasodilator mechanisms contribute to hypoxic cerebrovasodilation as well. Additionally, indirect evidence suggests opioid involvement in hypoxic cerebrovasodilation. For example, it was observed that hypoxia increases plasma methionine enkephalin in fetal sheep (75), plasma β-endorphin in human newborns at delivery (76, 77), and in those infants with hypoxic-ischemic encephalopathy with ongoing hypoxia (78). Most studies, however, have investigated mechanisms involved in hypoxic cerebro-vasodilation in the adult; mechanisms involved in hypoxic cerebrovasodilation in the newborn/infant period have received little attention.

Recently, it has been observed in the newborn pig that hypoxia is associated with elevated CSF levels of the opioids methionine enkephalin and leucine enkephalin, which are μ and δ opioid agonists, respectively. Because

μ and δ opioid receptor antagonists attenuated hypoxia-induced pial artery dilation (19, 20), these data further indicated that methionine enkephalin and leucine enkephalin contribute to hypoxic dilation. Using δ subtype selective antagonists it was observed that δ_1 receptor activation contributes to moderate and severe hypoxia (P_{ao2} of 35 and 25 mm Hg respectively)-induced vasodilation, but the δ_1 receptors appear to be more important during severe hypoxia relative to δ_2 receptors (20). Additionally, it was observed that δ_2 receptors primarily contribute to dilation during moderate hypoxia. Since the K_{ATP} channel antagonist glibenclamide attenuated hypoxic pial dilation (32) and activation of K_{ATP} channels also contributes to methionine enkephalin and leucine enkephalin dilation (32), these observations suggest that these two opioids contribute to hypoxia-induced pial artery dilation *via* activation of K_{ATP} channels. In contrast, hypoxic pial dilation was potentiated by the κ opioid antagonist, norbinaltorphimine (19), indicating that the endogenous κ agonist dynorphin opposes hypoxic dilation.

Since NO also contributes to hypoxia-induced pial artery dilation in the newborn pig (19), additional studies were designed to investigate the relationship between NO and opioids in hypoxic pial dilation. As described above, it has been observed that SNP and 8-Bromo cGMP produce large increases in CSF methionine enkephalin and leucine enkephalin concentration (19, 60). Since hypoxic increases in CSF concentration for these two opioids were attenuated by the NO synthase inhibitor L-NNA (60), these data suggest that NO contributes to hypoxic dilation, at least in part, *via* formation of cGMP and the subsequent release of opioids. L-NNA also blunted hypoxia-induced dilation that was further diminished by coadministration of L-NNA and the opioid antagonist β-funaltrexamine (19). Reversal of the above order of antagonist administration resulted in similar inhibition of hypoxic pial dilation (19). Therefore, hypoxic pial dilation is due to NO-induced release of opioids as well as to the direct action of NO.

Other mechanisms may also contribute to hypoxia-induced pial artery dilation. For example, the cyclooxygenase inhibitor indomethacin attenuates hypoxic hyperemia in piglets (67), indicating that prostaglandins contribute to hypoxic cerebrovasodilation. Therefore, studies were designed to determine the relationship between opioids and prostaglandins in hypoxia-induced pial artery dilation. Hypoxia-induced pial artery dilation was mildly attenuated during moderate hypoxia, while this response was blunted during severe hypoxia, by indomethacin (79). Hypoxic dilation was accompanied by increased CSF PGE_2 and 6 keto $PGF_{1\alpha}$ concentration; these two prostaglandins were observed to increase CSF methionine enkephalin and leucine enkephalin concentration. While indomethacin had no effect on the release of CSF opioids during moderate hypoxia, it attenuated the release of opioids during severe hypoxia (79). These data therefore suggest that elevated prostaglandin concentrations during severe hypoxia release opioids, which in turn contribute to hypoxic pial dilation. Additional studies were designed to determine the second messenger systems involved in the release of opioids by prostaglandins. As described above, both cAMP and cGMP are involved in the release of opioids in the piglet. Additionally, it has been observed that prostaglandin-mediated vasodilation is due to the release of NO and the production of cGMP and cAMP (80). By using probes for cGMP and cAMP function, it was recently observed that prostaglandin-associated elevated CSF cGMP and cAMP levels result in increased CSF methionine enkephalin and leucine enkephalin concentration (81). Therefore, prostaglandins can contribute to hypoxic pial dilation directly or indirectly *via* the release of opioids in a cyclic nucleotide-dependent manner.

Similar to prostaglandins, vasopressin has also been observed to contribute to hypoxic hyperemia in fetal sheep (74). Therefore, studies were designed to investigate the relationship between vasopressin and opioids in hypoxia-induced pial artery dilation in the newborn pig. Hypoxic pial dilation was attenuated during both moderate and severe hypoxia by a vasopressin receptor antagonist (82). Hypoxia-induced dilation was accompanied by increased CSF vasopressin concentration; exogenous vasopressin increased CSF methionine enkephalin and leucine enkephalin concentration (82). Furthermore, a vasopressin receptor antagonist attenuated the release of these two opioids during hypoxia (82). These data therefore suggest that elevated CSF vasopressin concentrations that occur during hypoxemia result in opioid release, which subsequently contributes to hypoxic pial dilation.

Finally, adenosine has previously been observed to contribute to hypoxic cerebrovasodilation in the adult rat (69). Using 8 phenyltheophylline and adenosine deaminase as probes for adenosine function, these observations were confirmed in the newborn pig (83). Additionally, it was observed that NO, cGMP, cAMP, and activation of K_{ATP} channels all contribute to adenosine induced pial dilation in the piglet (83). Having identified mechanisms involved in adenosine pial dilation, an attempt was then made to relate these data to the contribution of adenosine to hypoxic pial dilation. Since activation of K_{ATP} channels has been observed to contribute to hypoxic pial dilation (32), data from this study suggest that, at least in part, adenosine's role in hypoxic dilation involves the release of NO which activates K_{ATP} channels through a cGMP-dependent mechanism (83). It was initially hypothesized that opioids could serve as intermediates in this process since methionine enkephalin and leucine enkephalin contribute to hypoxia-induced pial dilation through activation of K_{ATP} channels (32). Addi-

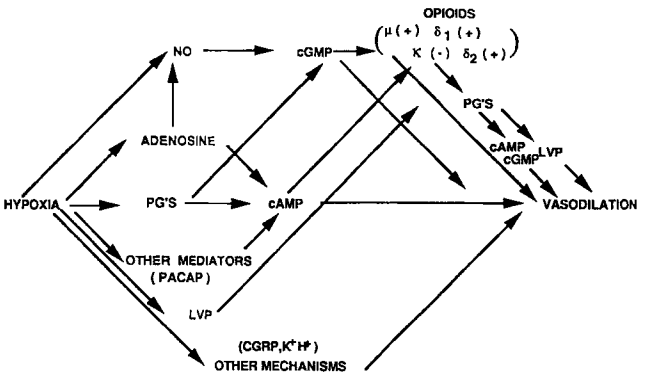

Figure 1. Interaction of opioids with other vasoactive systems in hypoxia-induced pial artery dilation. +, contributes; −, opposes; NO, nitric oxide; PG's, prostaglandins; LVP, vasopressin; PACAP, pituitary adenylate cyclase activating polypeptide; CGRP, calcitonin gene-related polypeptide.

tionally, cGMP has recently been observed to increase CSF methionine enkephalin and leucine enkephalin concentration (60). Therefore, adenosine could activate K_{ATP} channels *via* the sequential release of cGMP and opioids. However, results of this study show that adenosine does not alter CSF methionine enkephalin or leucine enkephalin concentration (83). The concentrations of adenosine used for investigation in this study were chosen based on their physiologic relevance. Using microdialysis, it has been observed that interstitial fluid adenosine concentrations in the frontal cortex of the newborn pig increased from 10^{-7} to 10^{-6} *M* during hypoxia while cisterna magna CSF levels increased from 10^{-8} to 10^{-7} *M* (84). Since concentrations of adenosine observed in response to hypoxia did not have any effect on CSF opioid concentration, these data indicate that adenosine and opioids contribute to hypoxic pial dilation independent of one another.

Figure 1 summarizes the role of opioids in hypoxic pial artery dilation in the newborn pig. In addition, this figure illustrates the role of other vasoactive systems important in hypoxic pial dilation and their relationship to opioids.

Role in Brain Injury. Previous studies have characterized the hemodynamic effects of brain injury in adult animals. While somewhat variable, most have observed reductions in cerebral blood flow and pial vessel diameter. For instance, decreased cerebral blood flow was observed in the adult monkey, cat, and rat (85–87), while elevated cerebral blood flow has been observed in the adult cat (88, 89). Such differences in cerebral hemodynamic response to brain injury could be caused by choice of anesthetic, species differences, or, more likely, a relatively different position on the stimulus-response curve. For example, Unterburg *et al.* (90) have observed that higher levels of brain injury in the adult cat were only associated with a reduction and not an increase in cerebral blood flow. However, few studies have investigated the effects of brain injury in the newborn-to-infant time period. Results of recent studies show that pial arteries constrict following brain injury in the newborn pig (91, 92). In addition, it has also been observed that developmental changes result in markedly different effects of brain injury on cerebral hemodynamics in the newborn and the juvenile pig (92). For example, the following were observed: (i) pial vessels constricted more and regional blood flow fell and remained depressed longer in newborn versus juveniles; (ii) there were marked increases in intracranial pressure in the newborn, but modest increases in intracranial pressure in the juvenile; (iii) there were differences in cerebral oxygenation, and index of metabolism: in the newborn saturation increased, followed by profound prolonged desaturation of hemoglobin for oxygen, while in the juvenile saturation increased modestly, followed by mild desaturation (92). Furthermore, systemic arterial pressure has been observed to increase in the adult studies (88) and in juvenile pigs (92), whereas systemic arterial pressure decreases following brain injury in the newborn pig (91, 92). These data suggest that cerebral and systemic hemodynamic responses following brain injury are age dependent. Because both newborn pigs and children less than 1 year of age have skulls with unfused sutures, the age period of pigs chosen in these studies may approximate the newborn-to-infant time period in the human. While many techniques have been used experimentally to investigate brain injury, it has been recently suggested that the lateral fluid percussion technique used in these studies may model many of the sequelae associated with closed head injury in the human (93).

Opioids are released following spinal cord injury and considerable evidence suggests that dynorphin is the predominant pathophysiologic opioid involved in spinal cord injury (94–96). These studies show that dynorphin is the only opioid observed to induce hindlimb paralysis when injected into the rat spinal cord, that κ opioid–selective antagonists reverse neurologic and histopathologic deficits associated with spinal cord injury, and that pre-treatment of animals with dynorphin antisera attenuates neurologic damage associated with spinal cord injury.

The putative role of opioids in the mediation of dysfunction after percussive brain injury has also been investigated. Hayes and colleagues (96) were the first to show that naloxone improved blood gas, EEG parameters, and brain perfusion pressure after fluid percussion brain injury in cats. These beneficial hemodynamic and neurologic effects of naloxone have been replicated in rats and recently naloxone has also been observed to improve long-term neurobehavioral outcome after brain injury in the rat (97). Regional increases in dynorphin immunoreactivity were found to correlate with local histopathologic damage and reductions in cerebral blood flow at 2 hr following brain injury in the adult

cat (98, 99). Since leucine-enkephalin–like immunoreactivity was unchanged and β-endorphin decreased, these authors suggested that activation of dynorphin and the κ opioid system could play a role in the injury process after brain trauma (100).

Further evidence in support of the pathologic role of κ agonists is found in the observations that central administration of dynorphin or the synthetic κ agonist U50,488H worsens neurologic outcome (101), while the more selective κ antagonists Win 44,441-2 and nalmefene improve regional blood flow, neurologic outcome, metabolism, and survival after brain injury (99, 102). Alternatively, U50,488H has been reported to improve spinal cord blood flow and neurologic recovery, after brain injury in mice (103). Further, Baskin *et al.* (104) reported that dynorphin improved survival but not neurologic recovery following middle cerebral artery occlusion in cats. Additionally, low doses of the nonselective opioid receptor antagonist naloxone have been observed to exacerbate outcome after brain injury (105). A partial explanation for these contradictory data could be that there are κ isoreceptors; different κ isoreceptors could mediate different physiological effects and the above drugs could have varying affinities for these receptors (106). Furthermore, μ receptor agonist release could be neuroprotective following brain injury and could account for naloxone's detrimental effects in the above study (105, 106). Thus μ and κ opioid receptor concentrations could be differentially regulated after fluid percussion brain injury (101). Finally, it should be noted that, in all of the above studies, tissue opioid concentration and receptor binding were determined; only one study has measured opioids in ventricular CSF after brain injury (107).

The CSF is a dynamic milieu capable of reflecting normal and pathologic processes. Opioids in CSF could therefore be used as an index of secondary injury subsequent to percussive brain insult. Recent studies in the newborn pig show that CSF opioid concentrations increase following brain injury and that the time course and relative increase in CSF concentration vary from opioid to opioid (91). While all CSF opioid concentrations increase with 10 min of injury, dynorphin demonstrated the largest proportional increase. Furthermore, the CSF concentration of methionine enkephalin, leucine enkephalin, and β-endorphin continued to increase over 180 min, while the dynorphin concentration progressively decreased with time (91). Because naloxone attenuates pial artery constriction and reductions in cerebral blood flow following brain injury, opioids contribute to altered cerebral hemodynamics after injury in the piglet (91).

Data from another study serve to partially explain how different opioid receptor subtype agonists can be either deleterious or beneficial following brain injury. Experiments were designed to investigate the influence of brain injury on opioid-induced dilation in response to physiological and pharmacological concentrations of endogenous opioids in cortical periarachnoid CSF. After brain injury, CSF methionine enkephalin concentration increases to $\sim 10^{-8}$ *M*, whereas leucine enkephalin and dynorphin increase to $\sim 10^{-10}$ *M* (91). Therefore methionine enkephalin and leucine enkephalin, endogenous μ and δ agonists, respectively, produce pial dilation that would be beneficial, serving as a physiological antagonist to brain injury-induced pial artery constriction. Although the CSF concentration of these two opioids is increased following brain injury, data from a recent study indicate that their beneficial role is decreased, since dilation and release of cGMP by these opioids is attenuated after brain injury (108). Moreover, brain injury reversed dynorphin from a dilator to a constrictor, further contributing to pial artery vasoconstriction following injury (108). Although the mechanism for this reversal is uncertain, there are at least three possibilities. First, dynorphin is a tone-dependent agent, eliciting dilation during normotension and constriction during hypotension (6). Because brain injury produces modest hypotension in the newborn pig, this could contribute to the reversal of dynorphin from a dilator to a constrictor. Second, brain injury–induced alteration of NO function could unmask a direct constrictor component of dynorphin due to unopposed activation of a different κ isoreceptor. Finally, these data could also be explained by the presence of different κ receptors on endothelial and smooth muscle cells. In that case, brain injury could selectively alter the ability of the endothelial κ receptor to release NO, leaving the smooth muscle κ constrictor receptor to act unopposed.

While the endogenous opioids methionine enkephalin, leucine enkephalin, and dynorphin are accepted by some authors (18), as somewhat selective μ, δ, and κ opioid agonists, respectively, others consider them quite promiscuous in their receptor interactions. While conclusions drawn concerning the role of opioid receptor subtypes based on results from these agents could therefore be somewhat controversial, the utility of these data is that they concern the actions of naturally occurring opioids. Such data therefore give physiological perspective to such studies. Recently, however, it was observed that brain injury attenuated pial dilation and the associated changes in CSF cGMP induced by the opioid receptor analogues DAMGO, DPDPE, and deltorphin (108), selective μ, δ_1, and δ_2 agonists, respectively (29, 31). Similar to dynorphin, brain injury also reversed the synthetic κ agonist U50,488H (25) from a vasodilator to a vasoconstrictor.

The mechanism for the altered cerebral hemodynamics observed after brain injury has been investigated previously. For example, functional alterations have been accompanied by abnormalities in endothelial morphology and impairment of endothelium-dependent re-

laxation (109, 110), suggesting that altered release of endothelium-derived relaxing factor contributes to the reduction in cerebral blood flow after brain injury. Intracellular generation of superoxide or other species could alter nucleotides, second messengers, receptors, and membranes, and the movement of superoxide anion out of the cell through anion channels could result in high concentrations of activated oxygen species at cell surfaces, including endothelium. Such oxygen species, then, may alter opioid-related nitric oxide generation, metabolism, or action. Brain injury has been reported to cause the generation of superoxide for at least 1 hour after injury (111). In that study, the sustained dilation and abnormal responsiveness of pial arterioles observed after injury could be reversed by treatment with the free radical scavengers SOD and catalase (111). Oxygen radicals also have been shown to increase blood-brain barrier permeability (112, 113), to produce ultrastructural changes in pial vessel endothelium (114), and to cause abnormal arteriolar reactivity (114). In the newborn pig, brain injury has also been observed to increase superoxide anion generation (115). Results of this study also show that the oxygen radical scavengers PEGSOD and catalase act on the cerebral vasculature of the newborn pig to improve altered vasculature reactivity after brain injury. Data from this study also show that pial vessels do not constrict as greatly after brain injury in animals pretreated with PEGSOD and catalase. These data therefore further suggest that superoxide anion contributes to altered opioid-induced cerebrovascular effects after brain injury possibly *via* interference with nitric oxide. Finally, altered cerebrovascular responsiveness following brain injury does not appear restricted to opioids since dilator responses to vasopressin are also reversed to constriction following injury (116).

Additional mechanisms for altered cerebral hemodynamics following brain injury have also been investigated. As described above, vasopressin contributes to β-endorphin–induced pial constriction and the constrictor potential for dynorphin (26, 48). Therefore, studies were designed to characterize the effect of brain injury on β-endorphin–induced constriction and the role of vasopressin in that constriction as well as in the reversal of dynorphin's vascular response following injury. It was observed that brain injury potentiated β-endorphin–induced pial constriction (117). Additionally, baseline CSF vasopressin concentration was increased following injury, while the stimulated release of vasopressin by dynorphin and β-endorphin was also enhanced (117). These data therefore show that vasopressin contributes to augmented β-endorophin pial constriction and the reversal of dynorphin's vascular effects following injury. Further, since CSF dynorphin and β-endorphin concentrations are increased following injury, these data suggest that these two opioids contribute to pial artery constriction observed following injury, at least in part, *via* the release of vasopressin.

Endothelin 1 (ET-1), a purported mediator of cerebral vasospasm, can be released by several stimuli, including vasopressin. A recent study was therefore designed to investigate the role of ET-1 in pial artery constriction and in the reversal of vasopressin from a dilator to a constrictor following brain injury (118). ET-1 elicited pial dilation at low concentrations and vasconstriction at higher concentrations. Brain injury reversed the dilation to constriction at the low ET-1 concentration and potentiated the constriction at high ET-1 concentrations (118). Brain injury markedly increased CSF ET-1 concentration and the ability of vasopressin to release ET-1. BQ123, an ET-1 antagonist, blunted pial artery constriction following brain injury and blocked the reversal of vasopressin from a dilator to constrictor after injury (118). These data show that ET-1 contributes to pial constriction following injury. They also indicate that vasopressin-induced release of ET-1 contributes to the reversal of vasopressin from a dilator to a constrictor following injury. Furthermore, these data suggest that elevated CSF vasopressin and ET-1 interact in a positive feedback manner to promote pial artery constriction following brain injury.

Since CSF dynorphin and β-endorphin concentrations increase following brain injury (91) and these opioids in turn are known to elevate CSF vasopressin concentration (26), dynorphin and β endorphin appear to play an important role in pial artery constriction observed following brain injury both directly and indirectly *via* their interactions with other vasoactive systems. The role of second messenger systems in the elicitation of vasoconstriction during pathophysiologic conditions has been previously described. The hydrolysis of PIP_2 *via* PLC generates the intracellular second messenger DAG and IP_3, which have been implicated as potent regulators of PKC and intracellular calcium release, respectively (119). Activation of PKC represents a direct means of eliciting vasoconstriction that does not require stimulation of myosin light chain kinase (120). It has been observed that PKC levels increase after brain injury and that inhibition of PLC improved metabolic and neurologic outcome after brain injury in the rat (121, 122). As described above, dynorphin and β-endorphin have been observed to elicit pial artery constriction *via* activation of PLC and PKC (55). Additionally, results of that study were the first to link these opioids with activation of PLC and PKC in brain injury induced pial artery vasoconstriction (55).

The role of opioids in pial artery constriction following brain injury is shown diagramatically in Figure 2. This figure also shows the relationship between opioids and other vasoactive systems in that constriction. It should be cautioned that these data were obtained from experiments using newborn pigs. The relationship of

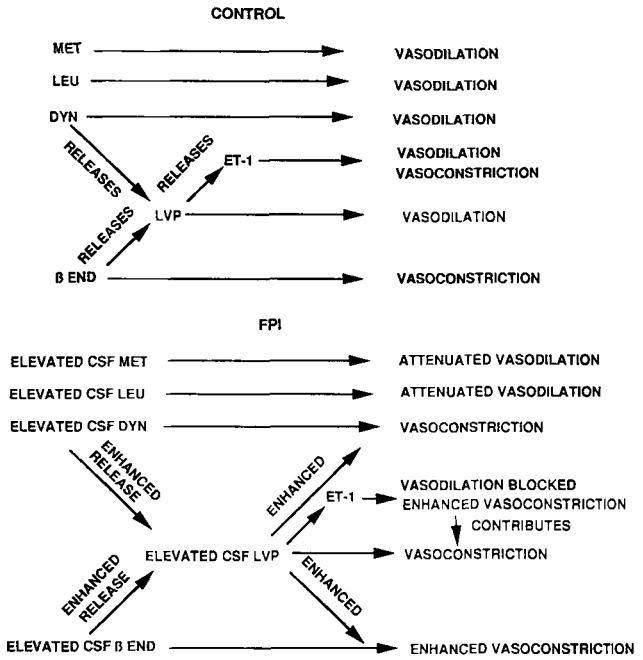

Figure 2. Interaction of opioids with other vasoactive systems under control conditions and after fluid percussion brain injury (FPI). Met, methionine enkephalin; leu, leucine enkephalin; dyn, dynorphin; β end, β endorphin; LVP, vasopressin; ET-1, endothelin-1.

these findings to the adult situation is therefore uncertain. The rationale for such newborn studies developed from the fact that traumatic injury is the leading cause of death for infants and children and that the presence of head injury greatly increases mortality (123). Unfortunately, little is known concerning the effects of brain injury on the newborn cerebral circulation or the mechanisms involved.

Concluding Remarks

Opioids are important in the regulation of the cerebral circulation. Their influence is subtle during physiologic conditions but more robust during pathophysiologic conditions. For example, opioids contribute to the control of cerebral hemodynamics during hemorrhagic hypotension, hypoxia, and brain injury. In many of these areas, opioids interact with other vasoactive systems in a complex manner resulting in a multifactorial response to pathologic stimuli.

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