Neuropeptides and Stroke: Current Status and Potential Application

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OVER THE PAST DECADE, more than 30 peptides have been identified in the central nervous system; these neuropeptides appear to subserve a role as both neurotransmitters and neuromodulators and have been found to be co-localized in many cases with "classical" neurotransmitters. The precise role(s) of these neuropeptides remains to be determined. Perhaps best established is the role of endogenous opiates (endorphins) and Substance-P in pain transmission. However, there is evidence to implicate peptides in the regulation of central cardiovascular and respiratory function, food and water balance, body temperature and memory. Moreover, there is recent evidence to suggest a role for endogenous opioid peptides in the pathogenesis of shock and spinal cord injury. The demonstration that the opiate receptor antagonist naloxone and the peptide thyrotropin-releasing hormone improve neurological recovery after spinal injury generated interest in the possible role of neuropeptides in the pathogenesis or treatment of other types of central nervous system injury. This paper summarizes the experimental and clinical evidence regarding the role of peptides in the pathogenesis and treatment of cerebral ischemia or infarction.

Endogenous Opioid Peptides

Discovery of the opiate receptor in 1973 led to the search for endogenous opioid substances. The pentapeptides methionine and leucine enkephalin were the first such substances to be identified. Subsequently, the list of endogenous opioids has been enlarged to include β-endorphin and dynorphin, as well as fragments of the β-endorphin precursor — β-lipotropin. Moreover, fragments of β-endorphin and dynorphin have been found which possess varying degrees of opioid activity.

This expansion in the number of recognized endogenous opioids has been accompanied by an expansion in the number of recognized opiate receptor subtypes. In addition, there is some support for the concept of high and low affinity receptor populations for several of these subtypes. Endogenous ligands have perhaps been identified for three of these receptors: enkephalins for the δ-receptor, dynorphin for the κ-receptor, and β-endorphin for the σ-receptor. But this area of research is moving very rapidly, and identification of further receptor subtypes and additional endogenous ligands appears almost certain. It also appears likely that the different receptors and ligands will be found to subserve different physiological roles.

In view of the enormous complexity of the endogenous opioid systems, one should be extremely cautious in proposing a role for "endorphins" or their receptors in the pathophysiology of a specific disease process. Similarly, one needs to be cautious in interpreting the results of studies utilizing only a single opiate receptor antagonist like naloxone. Although naloxone is relatively selective for the µ-receptor, at higher doses it will also antagonize effects at other opiate receptors. Moreover, despite the fact that naloxone has been called a "specific" opiate antagonist, it also has effects, particularly at high doses, which may not be opiate-receptor mediated. Recognition of these complexities and difficulties are particularly important in interpreting the results of experimental and clinical studies using naloxone in cerebral ischemia and infarction.

Opiate Antagonists: Experimental Studies

Hosobuchi, et al proposed that endogenous opioids might play a pathophysiological role in cerebral ischemia by showing that the opiate antagonist naloxone improved neurological function following carotid occlusion in the gerbil. In this study, homolateral ischemic injury was produced using unilateral, carotid artery ligation. Approximately 42% of the gerbils subjected to such ligation developed contralateral hemiplegia; in all gerbils tested, naloxone at a dose of 1 mg/kg administered intraperitoneally was said to fully reverse such hemiplegia. Unfortunately, this study suffered from several methodological problems, including an absence of true control animals and a lack of blinding among the investigators. Thus, two laboratories using similar techniques failed to reproduce the finding that naloxone has a beneficial effect in experimental cerebral ischemia in the gerbil. One such negative report has now been published.

We have approached this problem from a different perspective utilizing a different experimental model. Our investigation of naloxone in experimental stroke was based on our earlier observations that β-endorphin-like immunoreactivity was significantly elevated following experimental spinal cord injury and that naloxone significantly improved both spinal cord blood flow and subsequent neurological recovery. We felt that the pathophysiology of spinal cord injury and cerebral ischemia had many parallels. For example, the neurological deficit in both conditions may result from a critical reduction in blood flow below certain levels, and the pathophysiological consequences of both types of injury are potentially reversible for a period of time following injury. We therefore examined the ef-
fect of naloxone in experimental stroke,\textsuperscript{13} utilizing a canine embolic model developed by Hallenbeck, et al.\textsuperscript{14} Following one hour of ischemic injury, dogs received either naloxone at a dose of 2 mg/kg bolus followed by 2 mg/kg per hour, or physiological saline at equal volumes. Outcome measures included the cortical somatosensory-evoked response (CSER) and cerebral blood flow using $^{14}$-iodoantipyrine autoradiography. Naloxone-treated animals showed a rapid and significant improvement in the CSER over the hemisphere ipsilateral to the injury. Moreover, animals treated with naloxone did not show the multifocal areas of critically low blood flow characteristic of this air embolism model.\textsuperscript{13} Although our study did not examine the functional sequelae of the ischemia, the improvement in physiological variables following naloxone treatment supports the view that naloxone may be capable of limiting the extent of ischemic cerebral injury. Further support for this conclusion comes from a recent study by Zivin and colleagues (personal communication), which found that naloxone treatment protects rabbits from the paraplegia resulting from spinal ischemia produced by temporary aortic occlusion.

### Opiate Antagonists: Clinical Studies

Baskin and Hosobuchi reported that naloxone treatment, at low doses, completely reversed the neurological deficits in two patients suffering from cerebral ischemia, but failed to have a beneficial effect in another patient with demonstrated cerebral infarction.\textsuperscript{15} However, the two patients who were said to respond with naloxone had extremely complicated histories: both were post-operative following an intracranial procedure and showed diminished levels of consciousness. Thus it is difficult to interpret the reported beneficial effect of naloxone in these cases. Subsequently, preliminary findings from two other clinical studies were reported. Bredesen, et al found no effect from naloxone administration in ten unselected patients with acute vascular disease of the central nervous system.\textsuperscript{10} Jabaily and Davis observed partial recovery following naloxone administration in three of seven patients treated for cerebral ischemia or infarction.\textsuperscript{17}

There are major problems with each of the clinical studies reported to date. Patients were unselected, suffering from cerebral ischemia or infarction of varying etiologies. Furthermore, they were treated with only low doses of naloxone and treatment was usually not administered until many hours following the initiation of the neurological deficit. Under these circumstances, even if naloxone were therapeutically effective in acute stroke, it would be most unlikely to demonstrate a therapeutic benefit from such treatment.

The findings from experimental spinal injury indicate that very high doses of naloxone ($> 1$ mg/kg) are required to improve spinal cord blood flow and to prevent the neurological deficit.\textsuperscript{6, 18} Similar doses of naloxone have been required in experimental shock to improve tissue perfusion and survival.\textsuperscript{19-21} Because of such dose response data, we administered a high dose of naloxone in our canine stroke study. Thus, failure to observe a therapeutic response in stroke patients from a naloxone dose of 0.006 mg/kg, as reported, should not lead to the conclusion that naloxone treatment is ineffective in cerebral ischemia, rather that it may be ineffective at low doses. This is a point we have previously stressed with regard to clinical shock.\textsuperscript{22} Moreover, it makes little sense to evaluate the effects of naloxone treatment many hours or days after completed stroke. Again, it is clear from studies in experimental shock and spinal trauma that animals need to be treated with naloxone within the first hours following injury, that is, when the pathophysiological events are still reversible. Yet few of the naloxone-treated stroke patients reported to date have been started on therapy within the first hours following onset of neurological signs.

### Opiate Antagonists: Future Directions

The high doses of naloxone required in the experimental studies suggest that the beneficial effects of this opiate-antagonist may result from actions which are not opiate-receptor mediated. For example, naloxone can affect calcium flux and cyclic adenosine monophosphate, and at high doses may have antioxidant properties.\textsuperscript{9, 10} Alternatively, the high doses of naloxone needed may reflect actions at non-$\mu$, opiate-receptors. Such a conclusion is consistent not only with $\text{PA}_2$ studies,\textsuperscript{23} but with recent findings showing that a selective $\delta$-antagonist,\textsuperscript{24} but not a selective $\mu$-antagonist (in preparation), improves hemodynamic function in experimental endotoxin shock. That naloxone’s effects may be opiate-receptor mediated is supported by the observation that the beneficial effects of opiate-antagonists in experimental shock are stereospecific.\textsuperscript{20, 25}

These approaches need to be extended to studies of experimental stroke.

If further experimental studies support the conclusion that opiate-receptor antagonists are beneficial in stroke, pilot clinical studies should be undertaken which evaluate escalating-dose treatment with naloxone in a highly selected subgroup of patients with acute stroke. Preferably, such patients would include only those with acute cerebral ischemia of relatively short duration. Naloxone should be administered as an intravenous bolus followed by continuous infusion and should be evaluated at doses up to 1–2 mg/kg, if such doses appear safe in Phase I studies. This kind of approach is being followed in a clinical spinal injury study with naloxone, currently in progress at New York University Medical Center.

### Thyrotropin-Releasing Hormone (TRH)

TRH is a tripeptide which has been found to be widely distributed in the central nervous system and which has many physiological effects in addition to its endocrine functions.\textsuperscript{26, 27} One effect of TRH appears to be its ability to physiologically antagonize central autonomic actions of opioids without altering analgesia. These properties of TRH prompted us to examine its potential therapeutic actions in the same models in which naloxone had proved efficacious — experimental shock, spinal injury and stroke. Al-
though TRH proved to be superior to naloxone in shock and spinal injury, it failed to improve either the CSER or local cerebral blood flow in the canine model of embolic stroke. TRH has also proved ineffective in the gerbil model of ischemic stroke. However, recent evidence in the rat indicates that TRH improves spinal cord blood flow following traumatic injury and may be superior to naloxone in this regard (in preparation). In addition, high dose TRH-treatment has been reported to improve neurological function and the EEG following brain stem compression in the cat. Moreover, dose-response studies of TRH and TRH analogues in experimental spinal injury indicate that extremely high doses may be necessary to show a therapeutic effect (in preparation). Thus, the failure to observe a beneficial effect of TRH in experimental stroke may reflect the use of insufficient doses. Further experimental studies with TRH are clearly required before concluding that this agent has no therapeutic role in the treatment of stroke.

Vasoactive Intestinal Polypeptide (VIP)

VIP is one of a series of gastrointestinal hormones which are found in the central nervous system and which appear to act as neurotransmitters and neuromodulators. Immunoreactive VIP has also been found in nerve fibers supplying both extracranial and intracranial cerebral blood vessels. Larsson, et al first showed that VIP had a relaxing effect on cat cerebral arteries in vitro. VIP also produces dose-dependent increases in common carotid artery blood flow in the dog and causes dose-related vasodilation of pial arterioles in vivo and in vitro. Recently, Duckles and Said have demonstrated that in the absence of endothelial cells, cat cerebral vessels fail to relax to acetylcholine, but respond to both nerve stimulation and to VIP; these authors concluded that VIP might, therefore, mediate the non-cholinergic vasodilation observed in cerebral vessels. In addition to its effects on isolated cerebral vessels, it has been demonstrated that VIP, given intra-arterially, causes dose-dependent increases in cerebral blood flow in the goat and the baboon. Moreover, perfusion of cerebral ventricles with VIP causes a significant increase in regional cerebral blood flow. Taken together, these findings are consistent with the view that VIP may be locally released from perivascular nerve endings and under certain conditions may cause dilation of cerebral blood vessels and increases in regional cerebral blood flow.

To date, VIP has not been specifically implicated in the pathophysiology of cerebrovascular disease; nor has any therapeutic role for VIP been demonstrated in such conditions. Yet the observation of VIP immunoreactivity within cerebral vessels, combined with VIP's demonstrated physiological effects, calls for investigation of this peptide in pathophysiological studies of cerebrovascular disease.

Substance-P

Substance-P is another vasoactive peptide which has been found to be widely distributed both within the gastrointestinal tract and central nervous system. Like VIP, Substance-P has been identified, using immunocytochemical techniques, in cerebral vessels of a large variety of mammals. Similarly, Substance-P causes dilation of cerebral vessels both in vitro and in vivo. In addition, Substance-P has been shown to increase hypothalamic blood flow in the awake rabbit. Although Substance-P has not been specifically implicated in the pathophysiology of cerebrovascular disorders, the above findings suggest that a potential role for this peptide should be considered.

Other Peptides

A number of other neuropeptides have potent vaso-motor effects which may be centrally mediated and which may play a role in the pathogenesis or treatment of cerebrovascular disorders. These include neurotensin, angiotensin, oxytocin, vasotocin, vasopressin and corticotropin-releasing factor. Several of these have already been localized by immunocytochemical methods to cerebral blood vessels and have been implicated as playing a potential role in local vasoregulation.

Conclusions

Neuropeptide research is in an early stage of development. However, discoveries in this area have already led to new conceptualizations about the pathophysiology of disease states including neurological disorders. The current status and potential application of peptide research to the cerebrovascular area has been reviewed. Recent data suggest that a variety of neuropeptides may play a role in the regulation of cerebral blood flow. The potential application of these findings to research in cerebrovascular disease remains to be investigated.

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