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# Delta Opioid Peptide [*D*-Ala$^2$,*D*-Leu$^5$]Enkephalin Promotes Cell Survival

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**Key Words**

Opioid · Enkephalin · DADLE · Transplantation · Hibernation · Apoptosis · Methamphetamine · Dopamine · Ischemia · Reperfusion · PC12 cells · Neuroprotection

**Abstract**

By studying the hibernation in ground squirrels, a protein factor termed hibernation induction trigger (HIT) was found to induce hibernation in summer-active ground squirrels. Further purification of HIT yielded an 88-kD peptide that is enriched in winter hibernator. Partial sequence of the 88-kD protein indicates that it may be related to the inhibitor of metalloproteinase. Delta opioid [*D*-Ala$^2$,*D*-Leu$^5$]enkephalin (DADLE) also induced hibernation. HIT and DADLE were found to prolong survival of peripheral organs preserved en bloc or as a single preparation. These organs include the lung, the heart, liver and kidney. DADLE also promotes survival of neurons in the central nervous system. Methamphetamine (METH) is known to cause destruction of dopaminergic (DA) terminals in the brain. DADLE blocked and reversed the DA terminal damage induced by METH. DADLE acted against this effect of METH at least in part by attenuating the mRNA expressions of a tumor necrosis factor p53 and an immediate early gene c-*fos*. DADLE also blocked the neuronal damage induced by ischemia-reperfusion following a transient middle cerebral artery occlusion. In PC12 cells, DADLE blocked the cell death caused by serum deprivation in a naltrexone-sensitive manner. Thus, DADLE, and by extension the endogenous delta opioid peptides and delta opioid receptors, may play an important role in organ and neuronal survival. Here, critical developments concerning these fascinating cell protective properties of DADLE are reviewed.



## Background

Hibernation is a fascinating phenomenon in the nature. Animals that are hibernating exhibit profound physiological changes including respiratory depression, hypothermia, bradycardia, hypophagia, analgesia, and a cessation of renal output. These physiological changes reflect a tremendous reduction in metabolic rate which enables the hibernators to survive winters when food supplies are rare. Animals that hibernate include the ground squirrel, woodchuck, the brown cave bat, the European hedgehog, and the black bear.

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Studies of hibernation have led to the discovery that certain factor(s) in the plasma of winter hibernating animals may trigger hibernation. The transfusion of the plasma of hibernating thirteen-lined ground squirrels into either summer-active ground squirrels or woodchucks induced hibernation [11]. However, the purification and identification of the substance have been slow partly because no in vitro assay is available. At present, the only assay is the bioassay examining the induction of hibernation in summer-active ground squirrels or woodchucks. Despite this drawback, a protein factor that comigrates with serum albumin has been partially purified, which, when injected into summer-active ground squirrels, caused hibernation [7, 18]. Further purification of the hibernation induction trigger (HIT) has yielded an 88-kD peptide that is enriched in the plasma of the winter hibernators but not the summer-active animals [14]. Partial sequencing of the 88-kD peptide indicates that it has a high homology with a protein identified as human α1B-glycoprotein [15]. The function of α1B-glycoprotein is currently unknown. However, a metalloproteinase inhibitor homologous to α1B-glycoprotein has been identified. It is known that the European hedgehog, a hibernator, is resistant to the metalloproteinase present in the venom of the European viper [8]. It is not known at present whether the 88-kD represent a metalloproteinase inhibitor. The hibernation induction property of the 88-kD peptide has not been demonstrated in summer-active hibernators.

Since the physiological changes during hibernation carry a certain profile that is similar to that induced by opioids, vis-à-vis analgesia and respiratory depression, and since the endogenous opioid peptides have been found in the brain, it has been speculated that HIT may act like an opioid. Indeed, HIT-induced hibernation in ground squirrels was blocked by a universal opioid receptor antagonist naloxone [7]. HIT caused the depression of the electrically induced twitches of the guinea pig ileal myenteric plexus preparation. However, the depression caused by HIT could not be blocked or reversed by naloxone [7]. A potential explanation is that HIT is not opioid itself, but is a potent releaser of endogenous opioid peptides. Alternatively, the opioid-like substance in HIT is too small in amount to be detected by the guinea pig ileum bioassay (i.e., the naloxone reversibility cannot be detected or it was masked by other twitch-depressing substances). The 88-kD peptide, however, has not been tested in the guinea pig ileum bioassay.

The induction of hibernation by HIT and the reversal of the HIT by naloxone suggest that endogenous opioids and associated receptors may play important roles in hibernation. Direct infusion of opioids into summer-active ground squirrels indeed induced hibernation. However, different classes of opioids produced different profiles. Morphine and morphiceptin, relatively selective for mu opioid receptors, were low in efficacy in inducing hibernation [18]. Similarly, dynorphin and U-69593, two selective kappa opioids, were also low in efficacy in inducing hibernation in summer-active ground squirrels [17, 18]. However, morphine, morphiceptin, dynorphin, or U-69593, when coadministered with HIT, can block the HIT-induced hibernation [18]. On the other hand, DADLE, a selective ligand for delta opioid receptors, was high in efficacy in inducing hibernation [18]. The action of DADLE was like that of HIT [18]. DADLE did not block the hibernation induced by HIT. These results suggest that endogenous delta opioids and delta opioid receptors are important in the entry phase of hibernation and that mu and kappa opioids and associated receptors are important in the arousal phase of hibernation.

## Survival of Organs: The Peripheral Organ

Despite the long duration of hibernation which lasts usually about 5–8 months, animals arousing from hibernation exhibit no sign of damage to their internal organs after hibernation. The typical body temperature during hibernation is about 7 °C and the respiratory rate is about two respirations per minute [7, 18]. It is amazing that the organs of the hibernating animals can survive conditions of a near zero degree body temperature as well as hypoxia for such a long duration of time. Thus, there is a possibility that HIT or even DADLE cannot only induce hibernation but also promote organ survival.

A multiorgan preservation preparation has been found to prolong the organ survival. This preparation dissects the internal organs such as the heart, lungs, liver, spleen, jejunum and kidneys en bloc with veins and arteries connecting the organs [9]. The whole organs were preserved as a block in a preservation bath [9]. Compared to the conventional organ preservation, the multiorgan preservation preparation was able to prolong the survival of hard to preserve organs such as the heart and the liver from a survival time of about 2 h up to an average of 8 h. In this preparation, the heart is almost always the first organ to deteriorate. HIT, when injected into the multiorgan preservation preparation via veins, prolonged the survival of organs from 8 to 44 h [9]. When DADLE was administered to the multiorgan preservation preparation at 1 mg/kg (i.v.; original body weight) every 2 h, the organ

survival time was enhanced further to 46 h – the longest in the history of the preservation of the heart and the liver [10]. The lungs preserved with HIT in such a manner function normally when transplanted into a host animal [19].

The success of HIT and DADLE in promoting the survival of organs in the multiorgan preservation preparation suggested a possibility that perhaps they might be useful in prolonging organ survival even when each organ is preserved as an individual isolated unit.

DADLE enhances the survival of organs in single organ preservation preparation. Because of its delicate texture, the lung is one of the most difficult to preserve as a single organ. Lungs preserved with a standard Euro-Collins solution usually developed severe pulmonary edema, hemorrhage, and occlusive vascular resistance. However, DADLE, when added into the preservation buffer in a 24-hour hypothermic preservation of the rat lungs, dramatically promoted the survival of the isolated lungs [24]. The lungs thus preserved showed good air flow, almost normal vascular resistance, good oxygenation, and normal tissue wet/dry weight ratio [24]. These results indicate that DADLE substantially enhances the hypothermic preservation of the lung as an isolated unit.

HIT and DADLE also promote the survival of the isolated heart. Isolated rabbit hearts were prepared in the Langendorff fashion. Subjection of the heart to a global ischemia in a standard cardioplegic solution usually leads to only a 30% functional recovery of the heart. A preconditioning of the heart with HIT or DADLE for 15 min at 37°C before subjecting the hearts to 18 h of global ischemic storage at 4°C, however, increased the functional recovery of the heart to an average of 70% [2–4]. The isovolumin-developed pressure, maximal positive and negative dP/dt, coronary flow, and myocardial oxygen consumption were compared as a percentage of prestorage values versus 45 min after removal from storage and reperfusion. Interestingly, DPDPE, a selective delta-1 opioid, provides protective effects only on the developed pressure, positive and negative derivatives of left ventricular pressure and not on the coronary flow and the myocardial oxygen consumption [2–4]. Since DADLE is a nonselective peptide for delta-1 and delta-2 opioid receptors, it is possible that the myocardial protective effect exerted by DADLE is probably mediated via delta-1 and delta-2 opioid receptors. The results are in alignment with a later report that TAN-67, a delta-1 alkaloid opioid, elicited a cardioprotective effect via delta-1 opioid receptors [20]. The effect of TAN-67 was blocked by a selective delta-1 receptor antagonist [20].

## Survival of Organs: The Brain

The tissue-protective property of DADLE has been extended to the central nervous system. Methamphetamine (METH) is a drug of abuse which causes long-term loss of striatal (dopaminergic, DA) terminals after a high dose of single administration or a prolonged use at medium doses. DADLE, given intraperitoneally at 30 min before METH administration, completely blocked the DA transporter (DAT) loss induced by METH [22]. Further, DADLE, given 2 weeks after METH administration when the DAT had been reduced to an about 30% level, restored the DAT to a normal level [23]. The effect of DADLE against METH-induced DAT loss was at least in part mediated via opioid receptor [22]. However, other mechanism may be involved. METH neurotoxicity has been known to involve free radical formation. DADLE apparently acts as a free radical scavenger in sequestering the formation of superoxide anions and hydroxyl free radicals [22]. DADLE also prevents lipid peroxidation in the synaptosomal preparation [22]. The effect of DADLE against METH-induced effects may involve genomic interaction as well. The brain mRNA levels of an immediate early gene c-*fos* and the gene of a tumor necrosis factor p53 can be elevated by METH administration. DADLE abolished the elevated expression of the mRNA of the two genes caused by METH [12, 13]. These results indicate that DADLE can counteract the effects of METH-induced cellular damage even at the genomic level. It is possible, therefore, that the endogenous delta opioid system may represent one of nature's protective mechanism against cell death. It is interesting to note that endogenous opioid systems, specifically the delta opioids, have been implicated in the survival of animals against hypoxic shock [16].

Recent data have also indicated that DADLE can protect against ischemia-reperfusion-induced brain damage after transient middle cerebral artery occlusion [5]. Rats subjected to a 90-min unilateral middle cerebral artery occlusion followed by a 15-min reperfusion exihibited extensive infarction in the striatum. Administration of DADLE (i.p.; 4 mg/kg, 4 injections at 2-hour intervals) prior to the middle cerebral artery occlusion completely blocked the striatal infarction induced by the reperfusion [5]. Naltrexone, a universal opioid antagonist, only transiently blocked the early phase of the reperfusion-induced behavioral deficit but failed to block the prolonged protective effect of DADLE [5]. These results indicate that opioid receptors may be involved in the initial phase of the effect of DADLE but not the later phase.

DADLE also protects against the 6-OHDA-induced brain damage in a rat model of Parkinson's disease. Cultured fetal brain cells were stored over time with or without DADLE and then transplanted into the brain of 6-OHDA-treated rats. Grafted cells previously treated with DADLE promote markedly more robust behavioral recovery than control cells [11]. Moreover, animals pretreated with DADLE just prior to 6-OHDA lesion surgery exhibited a reduction in lesion severity of the tyrosine hydroxylase immunoreactivity [1]. These results suggest that DADLE may have a therapeutic potential for treating parkinsonism.

## Survival of Cells in Culture: Recent Progress

Cultured cells provide a useful tool to examine the mechanism(s) by which DADLE may exert its protective functions. DADLE promotes the survival of cultured fetal brain cells for transplantation [6]. Serum deprivation in PC12 cells induces cell death. DADLE-treated PC12 cells appeared morphologically similar to the cells that were treated with nerve growth factor [21]. The enhancement of PC12 cell survival by DADLE was blocked by 10 n*M* naltrexone [21].

## Conclusion

Thus, although the mechanism(s) of action of DADLE in protecting against organ and neuronal death needs to be fully clarified, DADLE appears to be an effective therapeutic agent for treating certain degenerative diseases both in the central nervous system and the peripheral system. Our results showing that DADLE, given 2 weeks after METH administration, was able to restore the loss of DA terminal damage induced by METH [23] indicate that DADLE is able to reverse the existing damage caused by prior insults. This will make DADLE an ideal therapeutic agent. It is not known at present whether the active substance in the HIT is a DADLE-like compound. The final purification and characterization of the active substance in 'HIT' will provide a direct answer. DADLE exerts these tissue-protective effects perhaps by interfering with the cell death pathway via, at least in part, the delta opioid receptor. A recent report has indicated that morphine, a mu opioid receptor agonist, induced Fas expression and promoted Fas ligand-mediated apoptosis [25]. It is likely, therefore, that different types of opioid receptors and their associated ligands may play as yet to be fully recognized roles in cell death and survival.

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