Central Non-Opioid Physiological and Pathophysiological Effects of Dynorphin A and Related Peptides

Vijay K. Shukla, Simon Lemaire

Department of Pharmacology, Faculty of Medicine, University of Ottawa, Ottawa, Ontario

Dynorphin A (Dyn A) and related opioid peptides derived from prodynorphin possess a high affinity for κ opioid receptors, but they also bind to other opioid receptors (μ and δ) as well as to some non-opioid receptor sites. Although the physiological role of these peptides is not well established, recent experimental data pinpoint their particular involvement in physiological and pathophysiological conditions that relate to algesia, spinal cord injury and epilepsy. In this paper, we review data which support the concept that the non-opioid behavioral effects of Dyn A and related endogenous peptides which are observed under these conditions are physiologically and pathophysiologically relevant.

Key Words: dynorphin, algesia, spinal cord injury, epilepsy, opioid receptor, NMDA receptor

INTRODUCTION

Dynorphin A (Dyn A), a 17 amino acid endogenous opioid peptide, was first isolated from porcine pituitary by Goldstein and colleagues in 1979. The peptide was identified as the most potent endogenous opioid compound in the peripheral guinea pig ileum (GPI) assay (Goldstein et al 1979; Chavkin et al 1982). Dyn A is derived from the processing of a polypeptide precursor known as prodynorphin (or proenkephalin B). Selective proteolytic cleavages of three regions of prodynorphin give rise to three distinct opioid peptides, namely α-neoendorphin, Dyn A and/or Dyn A-(1-8) and Dyn B. The processing of prodynorphin is tissue- and species-dependent and is regulated by the physiological and pathophysiological states of the animal (Weber et al 1982; Goldstein et al 1979; Kangawa et al 1979; Kilpatrick et al 1982; Millan et al 1986). Chemical and immunohistochemical studies have enabled the identification of dynorphin-like peptides in various tissues, including the brain, the hypothalamus, the spinal cord, the autonomic nervous system and the adrenal medulla (Goldstein and Ghazarossian 1980; Lemaire et al 1984).

Address reprint requests to: Simon Lemaire, Department of Pharmacology, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada K1H 8M5.

Dyn A and related peptides are widely distributed in the CNS. High concentrations of immunoreactive Dyn A were observed in the periaqueductal grey, limbic system and thalamus (Watson et al 1982; Khachaturian et al 1983; Millan et al 1984; Millan et al 1985). High concentrations of Dyn A were also found in the dorsal horn of the spinal cord (laminae I and V) (Basbaum and Fields 1984; Cruz and Basbaum 1985; Millan et al 1984). High levels of Dyn A were found in the adenohypophysis of rats (Seizinger et al 1984), although its levels in human pituitary were much less prominent (Gramsch et al 1982). In addition, there are strong parallels between the overall distribution of Dyn A in rat and human central nervous systems (Przewlocki et al 1980; Gramsch et al 1982; Czlonkowski et al 1983).

There is a relatively strong correlation between the distribution of central Dyn A and that of the κ opioid receptor for which the peptide displays a high affinity (Chavkin et al 1982; James and Goldstein 1984). High densities of κ opioid receptors are present in the hypothalamus periaqueductal grey, claustrum (Moris and Herz 1986) and spinal substantia gelatinosa (Czlonkowski et al 1983; Gramsch et al 1982; Pfeiffer and Herz 1981; Pfeiffer et al 1982). However, the physiological and pharmacological significance of such parallels have yet to be determined, since some areas of the brain, such as the periaqueductal grey, which contain both κ sites

and Dyn A do not respond well to exposure to κ opioid agonists (Jaffe and Martin 1990). In addition, *in vitro* binding studies indicate that Dyn A and related peptides possess a relatively high affinity for all opioid binding sites, including μ, δ and κ receptors (Quirion and Pert 1981; Garzon et al 1984; Landahl et al 1985). The possibility that these peptides bind to some non-opioid sites has also been suggested from the fact that the specific binding of [^{3}H]Dyn A is not totally displaced by opioid alkaloids (Smith and Lee 1988). Therefore, the physiological function of Dyn A and related peptides may depend upon their interaction with more than one type of receptor, and the factors which control the biosynthesis and release of these peptides may play an important role in determining their site of action and function.

Even though Dyn A and related peptides were the most potent endogenous opioid peptides in the peripheral GPI assay, they did not display any marked *in vivo* antinociceptive activity. Intracerebroventricular (i.c.v.) injection of Dyn A-(1-13) in rats and mice did not increase tail-flick latency to thermal pain stimuli, but rather reduced the analgesic activity of morphine and β-endorphin (Friedman et al 1981; Tulunay et al 1981). In contrast, intrathecal (i.t.) injection of the peptide produced hindlimb paralysis, an effect that obscured the interpretation of the analgesic activity of the peptide (Stevens and Yaksh 1986). The antagonism of morphine activity and hindlimb paralysis were not blocked by naloxone, indicating that opioid receptors are not involved in these effects of Dyn A. Moreover, [Des-Tyr^1]-Dyn A, a likely metabolite of Dyn A with no binding affinity for opioid receptors, was a potent blocker of morphine-induced analgesia and of hindlimb motor functions (Walker et al 1983; Faden and Jacobs 1984). Walker and colleagues (1982b) have demonstrated that within the Dyn A molecule two biological active sequences exist, one opiate and the other non-opiate, both of which are able to produce significant behavioral activity. The non-opioid effects were specific to Dyn A and related peptides, not being observed with U-50488H, a non-peptidergic κ specific receptor agonist (Stevens and Yaksh 1986; Jhamandas et al 1986). Therefore, although Dyn A was first defined as an opioid peptide, its main physiological and/or pathophysiological functions may be related to the stimulation of non-opioid Dyn-specific receptors. In this review, we focus on experiments that indicate that Dyn A and related peptides may play an important role in the etiology and/or modulation of algesia, spinal cord injury and epilepsy through their interaction with non-opioid receptors.

Algesia

Supraspinal antinociceptive effect of dynorphin

Since the isolation of Dyn A by Goldstein et al (1979), reports on the analgesic efficacy of this peptide have been controversial. Unlike other opioid peptides (enkephalins, β-endorphin), Dyn A and related peptides administered i.c.v. have been found to be ineffective when tested in thermal analgesic assays using heat, such as rat tail-flick tests (Walker et al 1982) and hot plate tests (Hayes et al 1983); whereas in non-thermal analgesic assays, such as the writhing test (Nakazawa et al 1985; Gairin et al 1988), the flinch jump response to foot shock (Petrie et al 1982) and the analgesic assays using mechanical painful stimuli (paw pressure, tail pinch) (Hayes et al 1983; Kaneko et al 1983), these peptides (i.c.v.) displayed a moderate activity. Since Dyn A and related peptides (i.c.v.) also induce various motor effects including "barrel-rolling," circling, jumping and ataxia in animals (Herman et al 1980), their analgesic activity in mechanical tests is questionable, although this activity has been mainly attributed to their selective interaction with κ opioid receptors (Smith and Lee 1988).

Supraspinal inhibition of μ receptor stimulation

The relative low analgesic activity of Dyn A and related peptides was initially ascribed to a putative rapid degradation by peptidases present at the site of injection (Chavkin et al 1982). However, it was subsequently found that these peptides possess potent antagonist activity against analgesia produced by μ receptor agonists. In rats, Dyn A-(1-13) (i.c.v.) antagonized morphine and β-endorphin induced analgesia in the thermal tail-flick assay (Tulunay et al 1981; Friedman et al 1981; Ren et al 1985). Such antagonism of morphine-induced analgesia has also been observed in rats after i.c.v. administration of the non-opioid peptide fragment [Des-Tyr^1]-Dyn A (Walker et al 1982a). These studies suggest that the supraspinal modulatory action of Dyn A and related peptides on opioid-induced analgesia is non-opioid in nature. The receptor involved in this non-opioid action has not yet been characterized. However, decreases in the concentration of μ receptors and increases in the level of immunoreactive (ir)-Dyn A-(1-8) have been observed in the brain of obese female Zucker rats which are hypersensitive to painful stimuli and resistant to the analgesic effects of morphine (Roane and Porter 1988; Roane and Porter 1986), suggesting a modulatory role for Dyn A-(1-8) on μ receptor expression and functions. In addition, the hypersensitivity of these rats to painful stimuli may indicate an algesic role for Dyn A and related peptides.

Spinal antinociceptive effect of dynorphin

Several investigators have also reported that Dyn A and related peptides are more effective in producing analgesia at the spinal level than at the supraspinal level (Nakazawa et al 1985; Piercey et al 1982; Przewlocki et al 1983a; 1983b). Intrathecal administration of Dyn A and related peptides produced analgesia against thermal nociceptive stimuli, although concomitant neurological impairments tend to complicate the interpretation of these findings (Han and Xie 1984; Herman and Goldstein 1985; Nakazawa et al 1985; Spampinato et al 1985; Przewlocki et al 1983a; 1983b; Faden

and Jacobs 1983; Stevens and Yaksh 1986). In another study, both Dyn A-(1-13) and Dyn A-(1-8) (i.t.) produced a biphasic analgesic response, with antinociception present 24 hours after the injection (Jhamandas et al 1986). The first phase was insensitive and the second phase sensitive to the opioid antagonist naloxone (Jhamandas et al 1986; Przewlocki et al 1983b; Herman and Goldstein 1985). Such biphasic analgesic response and prolonged analgesia were not observed with the non-peptidergic κ receptor agonist U-50488H (i.t.) (Jhamandas et al 1986). In contrast, Stevens and Yaksh (1986) reported that Dyn A, when administered i.t. at doses below those which affect motor functions and cause flaccid paralysis, does not produce any detectable antinociceptive activity. In another study, i.t. administration of Dyn A in rats resulted in an irreversible loss of the thermally evoked tail-flick reflex (Caudle and Isaac 1987). Pre-administration with an excitatory amino acid receptor antagonist (AP5) blocked the loss of tail-flick reflex (Caudle and Isaac 1988; Isaac et al 1990).

Spinal modulation of μ receptor stimulation

A modulation of morphine-induced analgesia was also observed at the spinal level, for example, after i.t. administration of Dyn A and related peptides. Both potentiating effects (Ren et al 1985; Jhamandas et al 1986) and attenuating effects (Schmauss and Herz 1987; Fujimoto et al 1990; Song and Takemori 1991) of Dyn have been reported. The potentiation of morphine-induced analgesia was observed with Dyn A-(1-13) and Dyn A-(1-8) and was reversed by naloxone, involving an opioid mechanism. The attenuating effect was specific to Dyn A (for example, the 17 amino acid peptide), whereas other Dyn-related peptides (Dyn A-(1-8), Dyn A-(1-13), Dyn A-(2-17), Dyn B and α- and β-neoendorphin), when administered i.t., did not have anti-analgesic activity even at high doses (Rady et al 1991). The nature of the receptor involved in the antianalgesic effect of Dyn A is unknown.

Spinal level of immunoreactive-dynorphin

In recent studies, immunoreactive (ir)-Dyn A has been monitored in the spinal cord in response to different types of noxious stimuli in an attempt to identify its possible physiological function. Rats subjected to the chronic pain of polyarthritis showed a pronounced increase in spinal prodynorphin mRNA and ir-Dyn A (Hollt et al 1987; Ruda et al 1988). The increase in the level of spinal Dyn A and α-neoendorphin was less significant upon attenuation of arthritic conditions (Millan et al 1986). The strong correlation between Dyn A levels in the lumbosacral cord and the severity of arthritic syndromes led these researchers to postulate that Dyn A plays a role in the regulation of chronic pain. In contrast, Faden et al (1985) reported that levels of ir-Dyn A are increased in the spinal cord after injury. These researchers have suggested a pathophysiological role for the peptide in the mechanisms of "spinal cord injury" (see below). These studies point to the difficulty in dissociating analgesic and motor effects of spinal Dyn in commonly used analgesic tests.

Synopsis

The effects of Dyn A and related peptides on algesia can be summarized as follows: 1. Dyn A and related peptides can decrease algesia (or produce analgesia) at both spinal and supraspinal levels; 2. the opioid analgesic effects of these peptides may be restricted to the mechanical and chronic types of pain through the stimulation of κ opioid receptors, their apparent analgesic activity in thermal pain stimuli being naloxone-resistant and mainly the result of a loss of tail-flick reflex and motor impairment; 3. Dyn A and related peptides can antagonize analgesia evoked by μ receptor agonists through the stimulation of non-opioid receptors; 4. Dyn A and related peptides may also induce and/or modulate algesia, decreasing the number and/or affinity of μ receptors and increasing the sensitivity of the animal (obese Zucker rats) to painful stimuli (see above).

Spinal Cord Injury

Role of dynorphin in spinal cord injury

Several immunohistochemical studies have revealed the presence of Dyn A and related peptides in the mammalian spinal cord (Botticelli et al 1981; Miller and Seybold 1987; Vincent et al 1982; Weihe et al 1985). Ir-Dyn A in the spinal cord of rats has been found to be elevated after spinal trauma, the increase in its concentration being directly related to the severity of the trauma (Cox et al 1985; Faden et al 1985). Using rats that were subjected to standardized impact trauma to the thoracic region of the spinal cord, Faden (1990) observed that treatment with Dyn A exacerbated the post-traumatic behavioral outcome, while treatment with Dyn A antiserum improved this condition. Moreover, i.t. injection of Dyn A into the spinal subarachnoid space produced a flaccid hindlimb paralysis in a dose-related manner in rats, an effect that can be compared with post-traumatic paraparesis in humans (Przewlocki et al 1983b; Faden and Jacobs 1984; Herman and Goldstein 1985; Spampinato and Candeletti 1985). The order of potency of endogenous Dyn-related peptides in producing motor impairment was as follows: Dyn A > Dyn A-(1-13) > α-neoendorphin = Dyn A-(1-8). The relatively high potency of these peptides in producing motor deficits supports the hypothesis that they function as a mediator in the pathogenesis of spinal cord injury (Faden 1990).

Dynorphin-induced reduction of spinal blood flow

In rats, Dyn A-(1-13)-induced hindlimb paralysis was accompanied by widespread neuronal injury in the lower thoracic and lumbosacral spinal cord (for example, at the vicinity of the i.t. site of injection) (Long et al 1988; Stewart and Isaac 1989). A correlation has been found between the

extent of nerve cell damage and grey matter necrosis and the degree and persistence of neurological deficits (Long et al 1988). One mechanism by which i.t. Dyn A-(1-13) induces a broad spectrum of neurological deficits and widespread neuronal injury may be through ischemic injury to the lumbosacral spinal cord. This possibility is supported by the observation that paralytic i.t. doses of Dyn A-(1-13) and Dyn A cause striking reductions in rat lumbosacral spinal cord blood flow (Long et al 1987; Thornhill et al 1989). In addition, the Dyn-induced reduction in blood flow was resistant to naloxone, indicating that this effect of Dyn was mediated by a non-opioid mechanism.

Non-involvement of opioid receptors

The opioid receptors (μ, δ, κ) did not appear to be involved in the neurological deficits observed after i.t. injection of Dyn A and related peptides (Herman and Goldstein 1985; Long et al 1988; Faden and Jacobs 1984). Dyn-induced motor dysfunction was not reversed or blocked by the opiate receptor antagonist naloxone. However, non-peptidergic κ-selective agonists did not produce motor dysfunction (Long et al 1988; Faden and Jacobs 1984). Paralysis was also produced by Dyn A-(2-17) (or [Des-Tyr^1]Dyn A) and Dyn A-(3-13), two non-opioid forms of dynorphin. One important characteristic of the opioid response to narcotic analgesics is a rapid development of tolerance after sustained exposure to the drug (Sharp 1984). However, the paralytic effect of Dyn A-(1-13) was not diminished after prolonged (seven days) i.t. infusion of the peptide (Long et al 1988a), suggesting that this effect is non-opioid in nature.

Partial involvement of opioid receptors

Resistance of Dyn A-induced hindlimb paralysis to naloxone and norbinaltorphimine has been found in some studies (Long et al 1988; Long et al 1989; Faden and Jacobs 1984; Herman and Goldstein 1985; Stevens and Yaksh 1986), but not in all studies (Przewlocki et al 1983b; Spampinato and Candeletti 1985; Spampinato et al 1988; Faden 1990). Some researchers have observed that naloxone and the κ selective opioid antagonist (-)-N-(3-furylmethyl)-α-normetazocene methanesulfonate (MR-1452) can block the motor effects of Dyn A (i.t.); tolerance to both antinociceptive and motor effects was also observed under specific experimental conditions (seven days i.t. infusion of low doses (1 nmol/μl; 0.9 μl/hr) of Dyn A with osmotic minipumps) (Spampinato et al 1988; Spampinato and Candeletti 1985). These apparent discrepancies may indicate that hindlimb paralysis results from the interaction of Dyn A with both opioid and non-opioid receptors. This suggestion is consistent with the observation that the non-opioid fragments of Dyn A — for example, Dyn A-(3-13), Dyn A-(2-17) and Dyn A-(2-13) are less potent than Dyn A in producing paralysis (Long et al 1988; Faden and Jacob 1984; Przewlocki et al 1983b). Alternatively, the need for high doses of naloxone to block Dyn A-induced paralysis may reflect a non-opioid protective function of naloxone (Curtis and Lefer 1980; Simpkins et al 1986; Koreh et al 1981). Naloxone has already been shown to produce non-opioid effects, including inhibition of proteolysis, stabilization of lysosomal membranes (Curtis and Lefer 1980), inhibition of neutrophil superoxide release (Simpkins et al 1986), inhibition of iron catalyzed liposomal lipid peroxidation (Koreh et al 1981) and reverse post-traumatic alteration of calcium and ascorbic acid homeostasis after spinal cord injury (Pietronigro et al 1985). Other opioid antagonists, such as the κ opioid receptor antagonist norbinaltorphimine, were inefficient in antagonizing the paralytic effects of Dyn A and related peptides (Faden 1990). Thus, the role of opioid receptors in the spinal neurotoxic effects of Dyn is not well supported by experimental evidence.

Excitotoxic effects of NMDA receptor stimulation

Over the past several years, it has been suggested that the excitatory amino acids L-glutamate (Glu) and L-aspartate (Asp) contribute to the pathogenesis of central nervous system injury through their action at the N-methyl-D-aspartate (NMDA) receptor (Lucas and Newhouse 1957; Olney et al 1971; Meldrum 1985; Choi et al 1988). Pharmacological blockade of this receptor improves behavioral outcome after spinal cord injury, whereas NMDA, but not its levoisomer, given prior to spinal cord injury significantly worsens behavioral outcome (Faden and Simon 1988). Treatment with NMDA antagonists also limits tissue damage after spinal cord trauma (Faden et al 1990). In addition, spinal cord trauma causes sustained decreases in total tissue levels of Glu and Asp (Demediuk et al 1989), as well as early transitory increases in extracellular concentrations of these amino acids (Panter et al 1990). Interestingly, i.t. administration of Dyn A also causes an early transitory increase in extracellular Glu and Asp (Oleshensky and Long 1991) followed by sustained decreases in total tissue contents of Glu, Asp and glycine (Gly) (at 24 hours), suggesting that excitatory amino acids may be involved in the neurotoxic response to Dyn A (Bakshi et al 1990; Erecinska et al 1984).

Role of the NMDA receptor in the neurotoxic effects of dynorphin

Recent studies indicate that Dyn-induced neurological dysfunction is antagonized by NMDA receptors antagonists. Treatment with competitive and non-competitive NMDA receptor antagonists blocked the loss of tail-flick reflex resulting from i.t. administration of Dyn A-(1-13) (Caudle and Isaac 1988; Isaac et al 1990) and attenuated Dyn A and Dyn A-(2-17)-induced hindlimb paralysis (Bakshi and Faden 1990a; 1990b). In addition, non-competitive NMDA receptor antagonists, such as MK-801, improved neurological and neuropathological behavioral outcome in animals treated with Dyn A (Rigamonti et al 1989). The mechanism by which the NMDA receptor can mediate the neurotoxic effects of Dyn A and related peptides remains speculative. The local

reduction in spinal cord blood flow subsequent to i.t. administration of Dyn A (Long et al 1987; Thornhill et al 1989) may induce spinal ischemia leading to secretion of Glu and Asp, overstimulation of the NMDA receptor, excessive entry of Ca^{2+}, increases in neuronal cell free fatty acid contents, destabilization of cell membrane network and neuronal cell death (Bakshi et al 1990). It is also possible that Dyn A causes the release of excitatory amino acids through pre-synaptic opiate receptor-mediated mechanisms independent of ischemia. Finally, Dubner and Ruda (1992) have proposed that Dyn A and related peptides facilitate the action of Glu on the NMDA receptor complex mainly through their interaction with non-opioid receptors.

Synopsis

The role that Dyn A and related peptides play in the development of spinal cord injury is supported by the following findings: 1. the spinal cord contains significant levels of ir-Dyn A; 2. the levels of spinal ir-Dyn A are increased in cases of spinal cord trauma, and the extent of the increase is proportional to the severity of the trauma; 3. spinal administration of Dyn A and related peptides cause motor impairment, flaccid paralysis and neurological damage at the site of injection; 4. the paralytic effects of Dyn A are not blocked by naloxone but are blocked by pre-administration of NMDA receptor antagonists; and 5. the paralytic effects are comparable to the excitotoxic effects of NMDA receptor stimulation, most likely being triggered by ischemia secondary to the reduction of spinal blood flow.

Epilepsy

Dynorphin-induced electroencephalographic seizures

Dyn A, when injected i.c.v. or into brain regions, produces motor dysfunction involving wild running, jumping, circling, barrel-rolling and ataxia (Herman et al 1980; Kaneko et al 1983; Nakazawa et al 1985; Nakazawa et al 1989). Naloxone-resistant EEG seizures have been observed in the cortex of rats and spontaneous epileptic mongolian gerbils after i.c.v. injection of Dyn A (Walker et al 1982; Lee et al 1983; Simonato et al 1987). A similar type of EEG seizure was also observed with [Des-Tyr^1]Dyn A (Walker et al 1982), suggesting the involvement of non-opioid mechanisms. In contrast, the Dyn A-induced EEG seizures observed by Simonato and colleagues (1987) were antagonized by pre-administration of β-FNA, an irreversible blocker of κ opioid receptors. β-FNA can also block other opioid and non-opioid receptors, by alkylation of non-specific membrane proteins (Leslie 1987). However, Ehlers (1984) reported that Dyn A (i.c.v.) does not induce EEG seizures in rats.

Anticonvulsive activity of Dyn A and related peptides

Different researchers have also reported that Dyn A and related peptides display anticonvulsant activity (Tortella 1988). Dyn A-(1-13) (i.c.v.) was found to elevate the threshold of flurothyl-induced seizures in rats, and this action was not reversed by naloxone (Tortella and Holaday 1986). An increase in seizure threshold has also been observed after i.c.v. administration of Dyn A-(3-13), a non-opioid form of Dyn A. However, Dyn A-(1-13), but not Dyn A-(3-13), non-competitively blocked the anticonvulsant effect of the μ-selective opioid agonist DAGO. In a similar study, i.c.v. injection of Dyn A-(1-13) and [D-Arg^2]-Dyn A-(1-13) reduced the clonic phase of pentylenetetrazol-induced seizures and this effect was antagonized by naloxone (Przewlocka et al 1983). In the same study, i.c.v. α-neoendorphin significantly facilitated clonic seizures but inhibited tonic seizures. Both effects were antagonized by naloxone, and the non-opioid peptide [Des-Tyr^1]-Dyn A had no anticonvulsive property.

Presence of Dyn A in the hippocampal mossy fibre system

The hippocampus has been implicated in the generation of epileptic phenomena in humans and in a large variety of animals models of epileptic seizures (Dichter and Ayala 1988). It appears that in the hippocampal mossy fibre system, Dyn A and related peptides are released from nerve terminals along with Glu and modulate synaptic activity through pre- and post-synaptic mechanisms (Chavkin et al 1983; Terrian et al 1988). Changes in hippocampal Dyn A content and distribution were observed in different experimental models of epilepsy. Decreases in the concentration of ir-Dyn were observed in the hippocampal mossy fibre system after amygdaloid kindling, electric shock seizures and kainic acid-induced seizures in rats and mice (Gall 1988; Iadarola et al 1986; Kanamatsu et al 1986a; 1986b). In rabbits, hippocampal Dyn A content was increased after amygdala kindling (Przewlocki et al 1983). The functional significance of such changes in the concentration of hippocampal Dyn A is not clear.

EEG seizures and alteration of the distribution of hippocampal Dyn A

Recent studies indicate that the redistribution of Dyn A in the various compartments of the mossy fibre system may be functionally more important than the change in its overall mean concentration. Limbic kindling and generalized seizures evoked by pentylenetetrazol and repetitive synchronous activation of the perforant path induced a synaptic reorganization of the mossy fibre pathway vis-a-vis the distribution of ir-Dyn A (Sutula et al 1988; Golarai et al 1988). Brain samples obtained from patients suffering from temporal lobe epilepsy displayed a reorganization in the location of ir-Dyn A in the hippocampal mossy fibre system, while control autopsy specimens did not (Houser et al 1990; de Lanerolle et al 1989). The functional significance of mossy fibre reorganization in human hippocampal formation depends, to a large extent, on the circuitry that is established. Since the mossy fibres are normally excitatory, a monosynaptic recurrent excitatory path would presumably

contribute to increased excitability and disynchronous firing of the granule cells and thus facilitate seizures. There is evidence that synaptic reorganization gives rise to abnormal activation of hippocampal pathways through an NMDA receptor-mediated mechanism (Mody et al 1988). The possible physiological role of Dyn A and related peptides in the reorganization of hippocampal circuits in epilepsy has evoked considerable interest.

Excitatory and inhibitory effects of hippocampal Dyn A and related peptides

Dyn A and related peptides have been shown to be released from mossy fibre nerve terminals along with Glu, and it has been postulated that they modulate synaptic activity through pre- and post-synaptic mechanisms (Chavkin et al 1983; Terrian et al 1988; Terrian et al 1989). Both excitatory and inhibitory effects have been ascribed to Dyn A in the hippocampus of control rats (Gruol et al 1983; Henriksen et al 1982; Moises and Walker 1985; Iwama et al 1986; McGinty et al 1983; Hoffman and Zamir 1984). Iontophoretic applications of Dyn A and related peptides to CA_1, and CA_3 hippocampal neurons of the rat were found to generate excitatory responses when single cell activity and hippocampal field potentials were recorded (Henriksen et al 1982). These excitatory responses were blocked by naloxone and Mg^{+2}, suggesting the involvement of both opioid and NMDA receptors. (The NMDA receptor is sensitive to the presence of Mg^{+2} in a voltage-dependent manner.) In a similar study (Moises and Walker 1985), Dyn A and related peptides also evoked a dose-dependent depression of both spontaneous and Glu-evoked discharge in a majority (63%) of CA_1 and CA_3 cells tested by a single unit extracellular recording technique. The latter effects of Dyn A were not blocked by naloxone. The excitatory response that was observed with a few cells was antagonized by naloxone. The authors explained the discrepancy between their results and those of Henriksen et al (1982) on the basis of a difference in sampling methods and the overall population of pyramidal cells to which the peptides were applied. All cells sampled in their study displayed significant levels of spontaneous activity or were driven with Glu, whereas Henriksen and colleagues (1982) confined their analysis of Dyn action primarily to slow-firing neurons rather than to normally quiescent cells. Conceivably, the effects of Dyn A and related peptides and the involvement of opioid and NMDA receptors in their action may differ in these two populations of pyramidal neurons. Dyn A also produced facilitatory and inhibitory effects on the number of spikes recorded in guinea pig hippocampal CA_3 pyramidal neurons *in vitro* using paired-pulse stimulation of the mossy fibres (Iwama et al 1986). These researchers speculated that μ and κ receptors were involved in these excitatory and inhibitory effects, respectively. However, they did not study the effect of naloxone on these responses.

Excitatory and inhibitory effects of Dyn A and related peptides in the substantia nigra

The presence of high concentrations of ir-Dyn A in the substantia nigra and the existence of a Dyn striatonigral pathway suggests the involvement of proDyn-derived peptides in the regulation of movement and motor functions at the level of basal ganglia (Khachaturian et al 1982; Vincent et al 1982; Weber et al 1982). Unilateral intranigral injection of Dyn A-(1-8), Dyn A-(2-17), Dyn A-(6-17) and enkephalins produced contralateral circling (Herrera-Marschitz et al 1984; Friederich et al 1987). The effects of Dyn A-related peptides were not blocked by naloxone, while those of enkephalins were naloxone-sensitive. These researchers have suggested that the involvement of Dyn A and related peptides in the control of nigral motor activity has both opioid and non-opioid components. Several lines of evidence also indicate that the substantia nigra facilitates the propagation of seizures in kindling and other seizure models (Iadorola et al 1986; McNamara et al 1984). Bilateral intranigral injections of Dyn A-(1-13) dose-dependently exerted seizure suppressant action in kindled rats, and this effect was not reversed by naloxone (Bonhaus et al 1987). In contrast, Garant and Gale (1985) observed naloxone-reversible anticonvulsant action of Dyn A-(1-13) against electric shock-induced seizures in rats after a bilateral intranigral injection.

Synopsis

The following major lines of evidence suggest that Dyn A and related peptides are involved in the pathophysiology of epilepsy: 1. Dyn A and related peptides are present in hippocampal mossy fibres and are released along with Glu upon electrical stimulation; 2. there is an alteration of the distribution of Dyn A in hippocampal mossy fibre system of epileptic patients and of animal models of seizure; 3. there is also a reorganization of hyppocampal mossy fibre pathways in epilepsy; 4. Dyn A and related peptides promote excitation and inhibition of hippocampal and nigrostriatal neurons; 5. the inhibitory effects are non-opioid, and the stimulatory effects are partly opioid as a result of their partial reversal with naloxone; and 6. the NMDA receptor complex is also involved in the excitatory effects of Dyn A and related peptides on hippocampal neurons.

CONCLUSION

The studies reviewed here indicate that Dyn A and related peptides play an important role in the physiology and/or pathophysiology of algesia, spinal cord injury and epilepsy. Most of these behavioral effects of Dyn A are resistant to naloxone and therefore do not involve the stimulation of opioid receptors. Dyn A and related peptides have been characterized as endogenous opioid peptides which possess some selectivity of action for the κ opioid receptor, but not with μ and δ opioid receptors. However, in studies of *in vitro* binding with membrane preparations of rat brain, the binding

of [^{3}H]Dyn A was not totally displaced by high concentrations of opiate alkaloids, but the remaining binding sites were displaced by Dyn A itself, indicating that the peptide possesses a non-opioid receptor (Smith and Lee 1988). In addition, the loss of tail-flick reflex, hindlimb paralysis and spinal reduction of blood flow were selective to Dyn A and related peptides, not being observed with opioid alkaloids. The fact that these peptides have no clear antinociceptive action against thermal pain stimuli and that their blockade of mechanical and chronic pain stimuli is weak compared with morphine or other opiate alkaloids suggests that their prime function is not to produce analgesia but rather modulate μ receptor-mediated analgesia, affect, motor and behavioral functions that involve their interaction with non-opioid binding sites.

The initial behavioral effects observed with Dyn A (i.c.v.) in mice possess some of the characteristics of convulsion produced by i.c.v. injection of NMDA, namely wild running, popcorn jumping and hindlimb jerking (Koek and Colpaert 1989; Shukla et al 1992). These excitatory effects of Dyn A and related peptides are not blocked by naloxone and norbinaltorphimine, but by non-competitive blockers of the NMDA receptor (Shukla et al 1992). The possible interaction of Dyn A and related peptides with the NMDA receptor is also supported by *in vitro* binding studies which indicate that the binding of [^{3}H]-Glu is displaced by Dyn A (Massardier and Hunt 1989).

The NMDA receptor is one of the binding sites for the excitatory amino acid neurotransmitters Glu and Asp. The NMDA receptor complex is linked to a Na^+/Ca^{2+} ion channel with a high Ca^{2+} conductance (MacDermott et al 1986), and the NMDA ion channel is subject to voltage-dependent Mg^{2+} blockade (Mayer et al 1984). The NMDA receptor is closely associated with a strychnine-insensitive glycine receptor (Johnson and Ascher 1987), which facilitates the opening of NMDA ion channel, and with phencyclidine (PCP) receptors (Lodge and Anis 1982), which are believed to be positioned within this channel (Kemp et al 1987) and allow PCP receptor agonists to perform an open-channel block. In addition, there is evidence that Zn^{2+}, acting at a separate site near the mouth of the NMDA ion channel, acts as an inhibitory modulator of the channel activity (Westbrook and Mayer 1987; Reynolds and Miller 1988). The site of action of Dyn A on the NMDA receptor complex is still not well-defined, but the fact that many of the non-opioid effects of this peptide are antagonized by competitive and non-competitive NMDA antagonists (Caudle and Isaac 1988; Long et al 1989; Bakshi and Faden 1990a; 1990b) combined with the direct competition of Dyn A with [^{3}H]Glu binding, indicate that Dyn A and related peptides may either potentiate the action of Glu on the NMDA receptor or act as an agonist on the NMDA receptor. In our laboratory, Dyn A did not affect the binding of the specific NMDA antagonist [^{3}H]CPP but competitively inhibited the binding of the PCP receptor ligand [^{3}H]MK-801, suggesting that the action of Dyn A is indirect and most likely the result of the interaction between the peptide and the PCP binding domain located inside the NMDA-linked ion channel (Shukla et al 1992). Binding of Dyn A to the PCP receptor may: 1. promote an open conformation to the channel after the interaction of Glu with the NMDA binding domain (Shukla et al 1992); 2. inhibit the uptake of Glu in a manner analogous to that of PCP on the uptake of neurotransmitters (Smith et al 1977; Bowyer et al 1984; Vignon and Lazdunski 1984; Johnson and Snell 1985); or 3. trigger the release of Glu from pre-synaptic nerve terminals which may possess the NMDA receptor (Faden 1992; Skilling et al 1992). Although the possible role for the NMDA receptor in the non-opioid effects of Dyn A is already well-documented, establishing the mechanism of action of the peptide at this level will require further study.

Therefore, the characteristics of the non-opioid receptor involved in the presumed physiological and pathophysiological functions of Dyn A and related peptides are still not well-defined. However, an indirect potentiation of the action of Glu on the NMDA receptor seems to be a promising area for future investigation.

ACKNOWLEDGEMENTS

We thank C. Lalonde for secretarial assistance. This work was supported by the Medical Research Council of Canada.

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