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Effects of microiontophoretically-applied opioid peptides on Purkinje cells in the cat cerebellum

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KEY WORDS opioid peptides; morphine; iontophoresis; Purkinje cells; cerebellum

ABSTRACT

AIM: The purpose of the present study was to examine the effects of microiontophoretically-applied opioid peptides on Purkinje cell of the cerebellum. **METHODS**: The effects of microiontophoretically-applied morphine, leucine-enkephalin (Leu-Enk), methionine-enkephalin (Met-Enk), and dynorphin 1 - 13 (Dyn) on the spontaneous discharge of Purkinje cells in the cerebellum of the anesthetized cat were examined. **RESULTS**: Microiontophoretic applications of Leu-Enk and morphine produced inhibitory and excitatory responses, respectively in Purkinje cells. Application of both morphine and Leu-Enk induced dose-dependent responses. The excitatory responses were antagonized by naloxone, whereas the inhibitory responses were not. Bicuculline, a GABA-A antagonist, completely abolished both the Leu-Enk- and morphine-induced-inhibitory responses. Iontophoretic application of Met-Enk and dyn produced inhibitory responses only. Met-enk- and dyn-induced inhibition was antagonized by naloxone. **CONCLUSION**: In Purkinje cell activity, microiontophoretically applied Leu-Enk- and morphine-induced excitation is connected with opiate receptors, whereas inhibition is related to the GABA receptor. However, Met-Enk and dyn produced only inhibitory effects via an opiate receptor in the cerebellum of cats.

INTRODUCTION

The existence of multiple opiate receptors is well established in the central nervous system[1-3]. Cerebellum membrane preparations of rabbit and guinea-pig have been found to contain large proportions of mu- and kappa-opioid binding sites, respectively[4,5]. Another report has demonstrated low levels of enkephalin and low density of stereospecific opiate binding sites in the cerebellum[6]. Sar *et al*[7] observed enkephalin-like immunoreactive cell bodies in the cerebellum of the rat which were identified as Golgi type-2 cells. In the cat, most enkephalin-like immunoreactive cells lie in the outer one-third of the granular layer, giving the appearance of a thin, regular layer close to the Purkinje cell layer. Thus, enkephalin may play a role in afferent and interneuronal cerebellar synaptic communication[8]. These findings suggest that endogenous opioid peptides may play an important physiological role in the cerebellum.

Purkinje cells in the cerebellum of the rat have been shown to be inhibited and excited by iontophoretic administration of normorphine[9]. Furthermore, receptor agonists have been observed to produce marked inhibition of firing of Purkinje cells in rat cerebellum[10,11]. We have previously demonstrated that microiontophoretic application or systemic administration of morphine produced inhibitory and excitatory responses in Purkinje cells in the cat cerebellum[12,13]. However, the role of microiontophoretic administration of opioid peptides in the spontaneous discharge of Purkinje cells has not yet been examined, and this was investigated in the present study.

MATERIALS AND METHODS

Experiments were performed on 43 cats of either sex, weighing 2.2 - 3.4 kg. Under ketamine (20 mg/kg, im)-induced anesthesia, a tracheotomy was performed. The femoral vein and artery were cannulated and the animal was then placed in a stereotaxis apparatus. Under further anesthesia with α-chloralose (60 mg/kg, iv), lobules VI-VII of the vermis were exposed by craniotomy. The exposed tissue was immersed in liquid

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paraffin and the temperature of the paraffin pool was maintained at 37 – 38 ℃. Animals were paralyzed with pancuronium bromide (Mioblock; initial dose 20 mg/kg, iv) and artificially respirated. Physiological conditions were maintained by monitoring blood pressure and end-tidal CO_2 (3.5 % – 4.5 %; 1H26 Nihondenki-San-ei Instrument Co, Ltd). Rectal temparature was also maintained at 37 – 38 ℃ by means of a heating pad.

Five-barreled glass micropipettes were used. The tip of the micropipette was broken back to a diameter of 5 – 10 μm, under microscopic control. The central barrel, used for the recording electrode, was filled with a 3 mol/L-NaCl solution, dissolved in 5 % fast green dye, to mark recording sites (resistence 20 – 40 MΩ). In the five-barreled micropipettes, three of the four side barrels were each filled with the following drugs; *DL*-noradrenaline hydrochloride 0.1 mol/L, pH 4.5 (Nakarai Chem), gamma-aminobutyric acid 0.5 mol/L; pH 4.5 (Tohyo Chem Ind), morphine hydrochloride 0.1 mol/L, pH 4.5 (Sankyo), naloxone hydrochloride 0.1 mol/L, pH 4.5 (Endo Laboratories Inc), leucine-enkephalin (Leu-Enk) 0.02 mol/L, pH 4.5 (Sigma), methionine-enkephalin (Met-Enk) 0.02 mol/L, pH 4.5 (Sigma), and dynorphin 1-13 Dyn 0.01 mol/L, pH 4.5 (Sigma). The remaining side barrel, the balance barrel, was filled with a solution of 2 mol/L-NaCl. Substances were applied from the micropipettes adjacent to the recording site by microiontophoretic application, which was performed using a constant-current pump (Iontophresis Pump Neuro Phore BH-2, Medical System Co), with the retaining current being 10 – 15 nA. All substances were injected as cations.

Single-unit spontaneous activity of Purkinje cells was recorded from the vermis (lobules VI-VII) in the cerebellar cortex. Extracellular recording was performed via the electrode, which was connected to a preamplifier. Spike potentials in the Purkinje cells were measured by means of a window discriminator. Electrical activity was displayed on a medical oscilloscope with an audiomonitor. A signal processor (Model 7T08, Nihondenki San-ei Instrument Co, Ltd) was used for compiling the data in the form of pulse density variation histograms. The spontaneous activity of each cell was monitored in the first instance for 10 – 15 min to ensure a stable baseline before recording began. The effects of the previous drug on the spontaneous discharge were observed after ejection for 60 s. The next drug was ejected 1 – 3 min after recovery of the responses. The actions of opioid were scored when the spontaneous activity changed the pre-drug firing rate by at least 30 %. Antagonistic actions on opioid-induced responses were scored as significant when the response was reduced to at least 50 % of control values.

After the experiments, the position of the electrode tip on the cerebellum was verified. A negative current of 20 nA was applied for 15 – 20 min through the central barrel, which was filled with fast green dye. Animals were sacrificed by intravenous injection of pentobarbital-Na. The cerebellum was removed and fixed in 10 % formalin. Several days later, the cerebellum was cut into 60 μm sections using a freezing-microtome to determine the recording site.

Statistical analysis was performed using specify whether paired or non-paired *t*-test for significant differenes and the data were presented as $\bar{x} \pm s$.

RESULTS

Identification and characterization of Purkinje cells Spontaneous discharge of the Purkinje cells consisted of simple and complex spikes with a regular firing rate of 30 – 50 spikes/s and an average firing rate of (43 ± 28) spikes/s. The complex spike was further identified through observation of the climbing fiber response elicited by stimulation of the inferior olive nucleus[14].

Effects of opioid peptides on spontaneous discharge of Purkinje cells The responses of 296 Purkinje cells to microiontophoretic application of opioid peptides in three incremental currents (50, 100, and 150

Tab 1. Neuronal responses to iontophoretically-applied morphine and leucine-enkephalin in cerebellar Purkinje cells.

Drugs	Ejection currents /nA	Total number of cells	Number of cells exhibiting opioids-induced responses /%		
			Excitation	Inhibition	No effect
Morphine	50	46	11(24)	14(30)	21(46)
	100	48	13(27)	17(35)	18(38)
Leucine	50	26	1(4)	10(38)	15(58)
Enkephalin	100	55	8(14)	19(35)	28(51)

Tab 2. Neuronal responses to iontophoretically-applied methionine-enkephalin and dynorphin 1 – 13 in cerebellar Purkinje cells.

Drugs	Ejection currents /nA	Total number of cells	Number of cells exhibiting opioids-induced responses / %		
			Excitation	Inhibition	No effect
Methionine	50	41	0(0)	21(51)	20(48)
Enkephalin	100	34	0(0)	19(57)	15(43)
Dynorphin 1 – 13	50	21	0(0)	16(76)	5(24)
	100	25	0(0)	20(80)	5(20)

nA) were investigated. Tab 1 and 2 show the neuronal responses to ejected opioids. Spontaneous discharges were excitatory (increase in firing rate) and inhibitory (decrease in firing rate) in response to microiontophoretic application of Leu-Enk and morphine. Low level current (50 nA) applications of morphine altered the spontaneous discharge of 25 out of the 46 (54 %) Purkinje cells tested. Fourteen of the 46 cells showed inhibitory responses to morphine, the remainder showed excitatory responses. Microiontophoretically-applied Leu-Enk (50 nA) altered the spontaneous discharge of 11 of the 26 (42 %) Purkinje cells tested. Ten of the 26 Purkinje cells showed inhibitory responses to Leu-Enk, whereas 1 out of the 26 cells showed excitatory responses. High-level current (100 nA) application of morphine altered the spontaneous discharge of 30 out of the 48 (62 %) Purkinje cells tested. Seventeen out of the 48 Purkinje cells showed inhibitory responses to morphine, whereas 13 of the 48 cells showed excitatory responses (Tab 1). Fig 1 shows a representative record illustrating the dose-dependent excitation and inhibition of sponaneous discharge of the Purkinje cells by morphine applied at 50, 100, and 150 nA current. Leu-Enk (100 nA) altered the spontaneous discharge of 27 out of the 55 (45 %) of the Purkinje cells tested; inhibition occurred in 19 of the 55 cells, and excitation in 8 of the 55 cells (Tab 1).

Effects of microiontophoretically-applied Met-Enk and Dyn were studied in 121 Purkinje cells. The results demonstrated that iontophoretically-applied Met-Enk and Dyn induced only inhibitory responses (Tab 2). Low level current (50 nA) application of Met-Enk altered the spontaneous discharge of 21 out of the 41 (52 %) Purkinje cells tested by inducing an inhibitory response. Microiontophoretically-applied Dyn (50 nA) altered the spontaneous discharge of 16 out of the 21 (76 %) Purkinje cells tested by inducing an inhibitory response. High level current (100 nA) application of Met-Enk altered the spontaneous discharge of 19 out of the 34 cells (57 %) Purkinje cells tested by inducing an inhibitory response. Furthermore, Dyn (100 nA) altered the spontaneous discharge of 20 out of the 25 (80 %) Purkinje cells tested by inducing an inhibitory response.

Antagonistic effects of naloxone on opioid peptides-induced excitatory and inhibitory responses To determine the specificity of the opioid peptide effects with respect to mediation by the opiate receptor, naloxone was applied concurrently with the opioids on to the Purkinje cells. Naloxone (30 nA) antagonized morphine (100 nA)-induced excitation in all cells (8 cells). On the other hand, no antagonism by naloxone of the inhibitory effects of morphine (100 nA) on Purkinje cells (7 cells) was observed. Fig 2 illustrates the antagonism by naloxone of the Leu-induced responses. Naloxone (30 nA) antagonized the excitatory effect of the Leu-Enk (100 nA) in 5 out of the 5 (100 %) Purkinje cells. However, the inhibitory effects of Leu-Enk were not affected by naloxone (Fig 2). Microiontophoretically-applied Met-Enk and Dyn showed only inhibitory responses. Naloxone antagonized Met-Enk (50, 100 nA) and Dyn (50, 100 nA) -induced inhibition in all cells (Fig 3).

Naloxone (30 nA) alone did not affect spontaneous firing of Purkinje cells (Fig 2). The inhibitory response of the Purkinje cells to GABA was not affected by microiontophoretic administration of naloxone (30 nA) (Fig 2).

Antagonistic effects of bicuculline on morphine and Leu-Enk-induced inhibitory responses The inhibitory effect of morphine (100 nA) on the Purkinje cells was antagonized by bicuculline, a GABA antagonist (Fig 4). On the other hand, no antagonism by bicuculline (30 nA) of the excitatory effects of morphine (100 nA) on Purkinje cells (5 cells) was observed (Fig 4). The excitatory effects of Leu-Enk and morphine were not affected by iontophoretic application of

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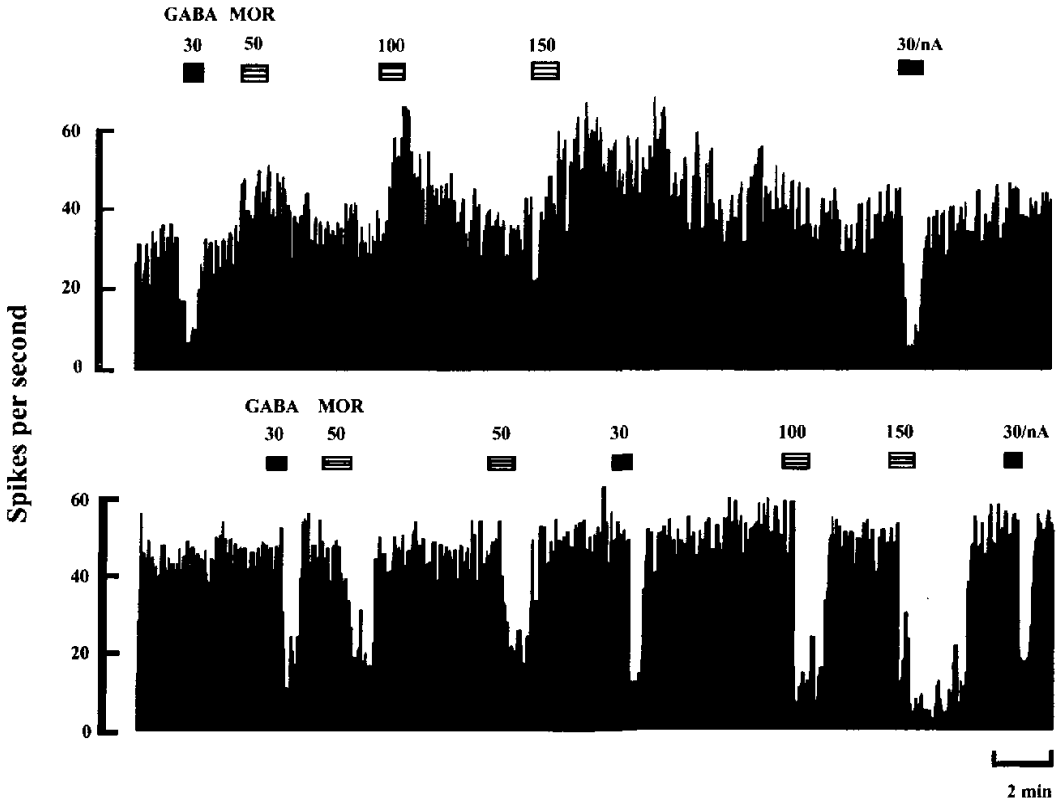

Fig 1. Effects of microiontophoretic application of morphine on the spontaneous discharge of a Purkinje cell in the cat cerebellum. Frequency histogram of the firing rate for morphine (MOR)-induced excitatory and inhibitory response. Note that the effects at each dose of morphine (50, 100, and 150 nA) resulted in a dose-dependent excitation and inhibition of spontaneous activity of Purkinje cell. The duration of ejection of drug is indicated by the horizontal bars above each record; numbers directly above each bar refer to the iontophoretic current in nA used for the ejection of drugs. The abscissa shows the rate for ejection of drugs. The ordinate shows the firing rate in spikes per second. The time bar is 2 min.

bicuculline. Bicuculline (30 nA) alone resulted in a slight increase in spontaneous firing in all the 18 cells tested. The GABA-induced inhibitory response was completely antagonized by microiontophoretically-applied bicuculline (Fig 4).

DISCUSSION

In the present study, we observed that microiontophoretic applications of both Leu-Enk and morphine increased, as well as decreased the spontaneous firing rate of Purkinje cells in the cerebellum. In contrast, iontophoretic applications of Met-Enk and Dyn showed only a decreased response. We have previously reported that microiontophretically- and systemically-applied morphine exhibits two types of response (both increase and decrease in the firing rate) on Purkinje cells in the cat cerebellum[12,13]. Firing of the Purkinje cells in the rat cerebellum have been reported to be both decreased and increased by normorphine, with both effects being antagonized by naloxone[9]. In the present study, naloxone failed to reverse the inhibitory responses of Leu-Enk and morphine, whereas the excitatory responses were antagonized by microiontophoretic administration of naloxone. However, Met-Enk and Dyn-induced inhibitory responses were antagonized by naloxone. Thus, inhibitory responses of Met-Enk and Dyn on the cerebellar Purkinje cells were affected by the opiate receptor. On the other hand, Leu-

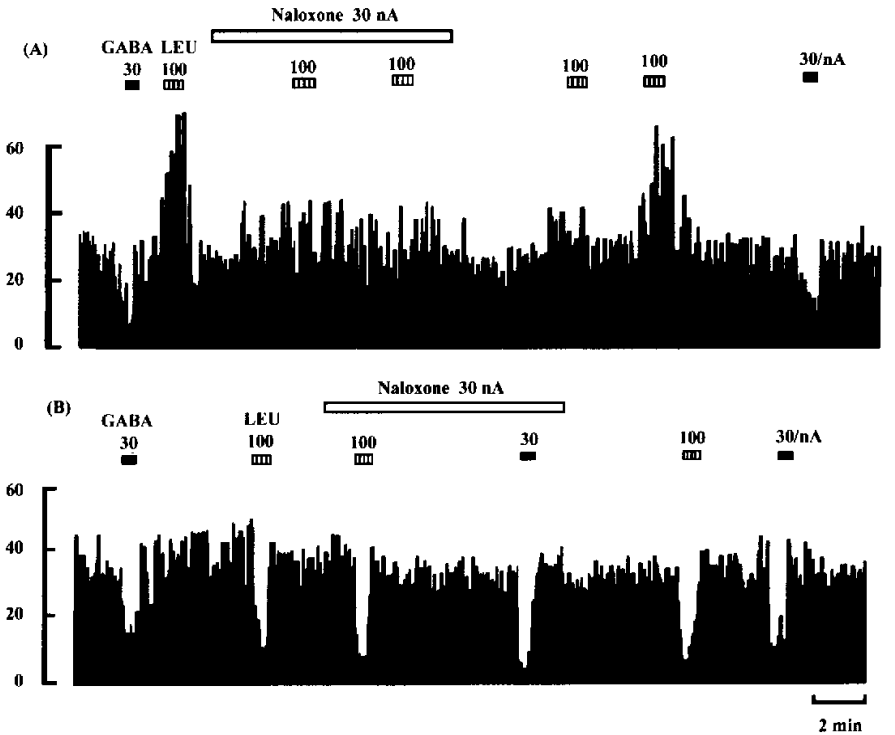

Fig 2. Frequency histogram illustrating the effects of naloxone on Purkinje cell responses to leucine-enkephalin (LEU). Naloxone (30 nA) completely antagonized the leucine-enkephalin (100 nA)-induced excitation (A), but failed to antagonize the leucine-enkephalin-induced inhibition (B).

Enk- and morphine-produced inhibitory responses may not be affecting the opiate receptor, but may instead be producing an interaction between other receptors, thus, the inhibitory effects of Leu-Enk and morphine on Purkinje cells may be mediated by receptors other than opioid. These results are in agreement with earlier reports on antagonism of morphine-induced inhibition in Purkinje cells by iontophoretically applied bicuculline and picrotoxin, as GABA antagonists[13]. Furthermore, Purkinje cells are known to receive inputs from the basket cells containing GABA which may act as an inhibitory transmitter. Werz and MacDonald[15] have shown by intracellular recording in cultured murine spinal cord neurons that morphine was directory antagonized by the postosynaptic actions of GABA. In addition, inhibitory responses in Purkinje-cell-firing induced by microiontophoretic applications of GABA were completely blocked by systemic administration of morphine[16]. In receptor binding studies, large concentrations of opiates were observed to displace the binding of GABA receptors[17]. Acute administration of morphine produces a decrease in the binding of GABA in the cerebellar cortex and striatum. Thus, morphine may produce some of its effects by modulating the GABAergic system[18]. The above findings suggest that morphine interacts likely with the GABA receptor in the cerebellum. In our experiments, the inhibitory effects of microiontophoretically applied Leu-Enk and morphine were blocked by bicuculline, a GABA receptor antagonist. Leu-Enk- and morphine-induced inhibition may thus occur through an interaction with GABA receptors in the cerebellum. In the present study, iontophoresis of naloxone antagonized the excitatory effects of Leu-Enk and morphine. A previous study demonstrated that naloxone was capable of blocking a morphine-induced increase in firing of the hippocampus[19] in rabbits, however, these findings did not exclude the possible existence of a disinhibitory mechanism. Previous studies on hippocampal pyramidal cell activity have reported predominantly excitatory effects of opioid peptides and of opiates, but these effects are presumably mediated through a disinhibitory mechanism[20, 21]. Such disinhibitory effects, resulting from an antagonism of GABA-mediated synaptic

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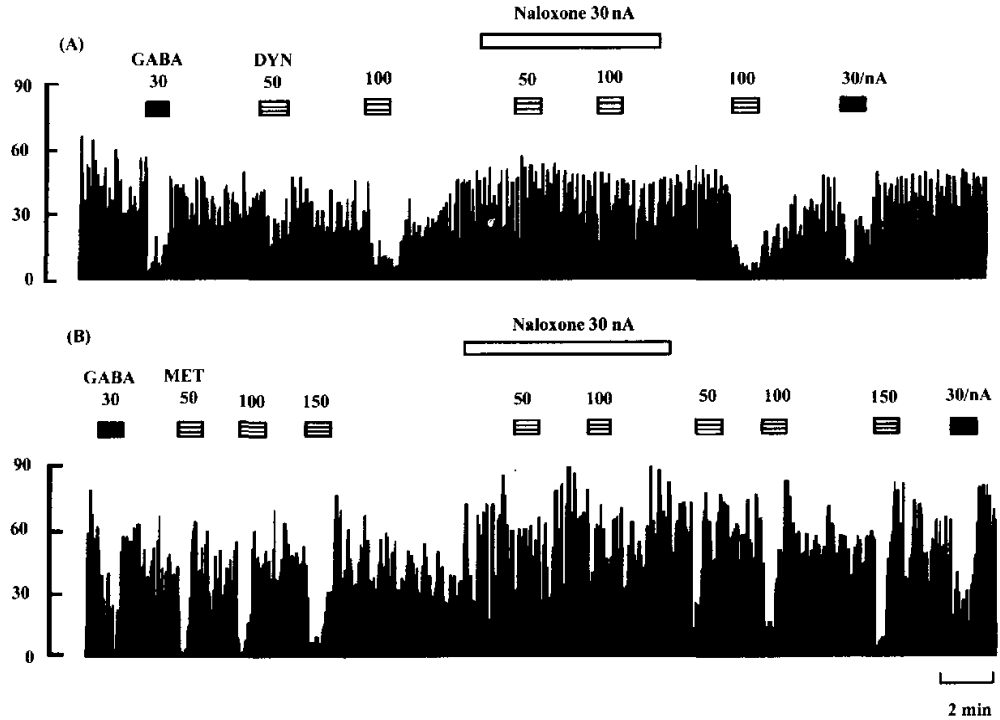

Fig 3. Frequency histogram illustrating the effects of naloxone on Purkinje cell responses to dynorphin 1 – 13 (DYN) and methionine-enkephalin (MET). Naloxone (30 nA) completely antagonized dynorphin 1 – 13 (100 nA) * (A) and methionine-enkephalin (100 nA) -induced inhibitory responses (B).

inhibition, have been shown to underlie some of the convulsant and excitatory actions of both moderate[19] and relatively high doses of opiates[1, 22]. Therefore, it is possible that the excitatory effects of Leu-Enk and morphine may be related to disinhibition of interneurons through an opiate receptor in the cerebellum.

In conclusion, the present results suggest that iontophoretically induced excitation of Purkinje cells in the cerebellum by morphine and opioids is associated with the opiate receptor, whereas inhibition with the GABA receptor.

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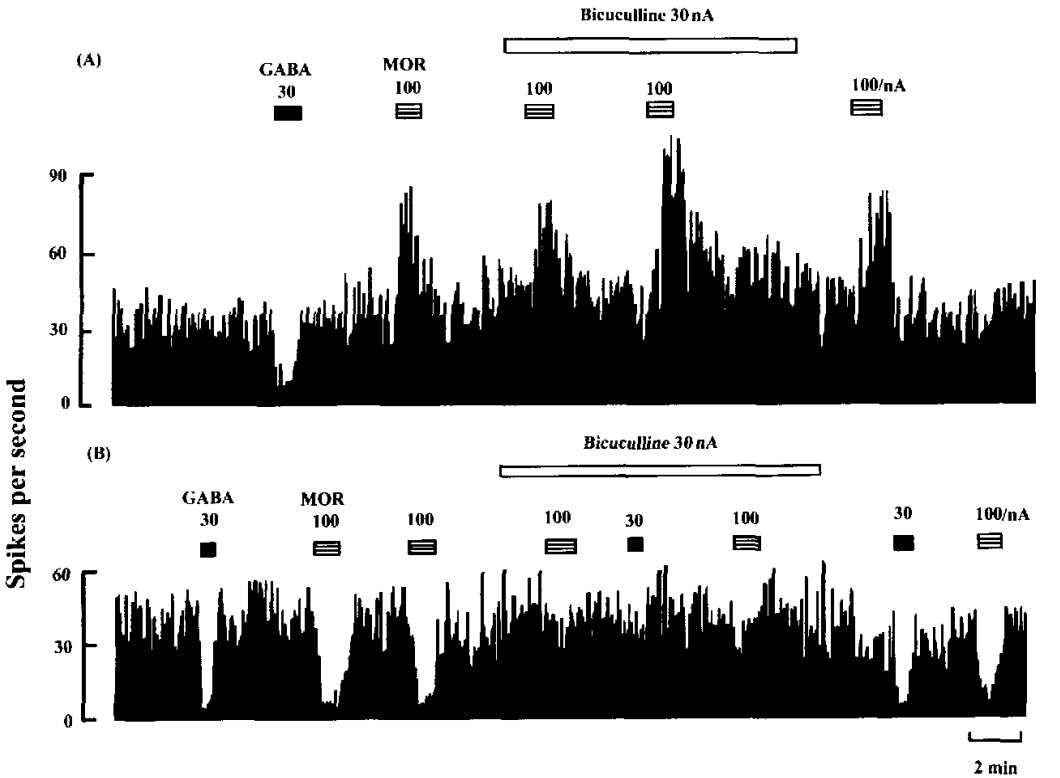

Fig 4. Frequency histogram illustrating the effects of bicuculline, a GABA antagonist, on Purkinje cell responses to morphine (MOR). Bicuculline (30 nA) failed to antagonize morphine (100 nA)-induced excitation (A), but completely antagonized morphine(100 nA)-induced inhibitory responses (B).

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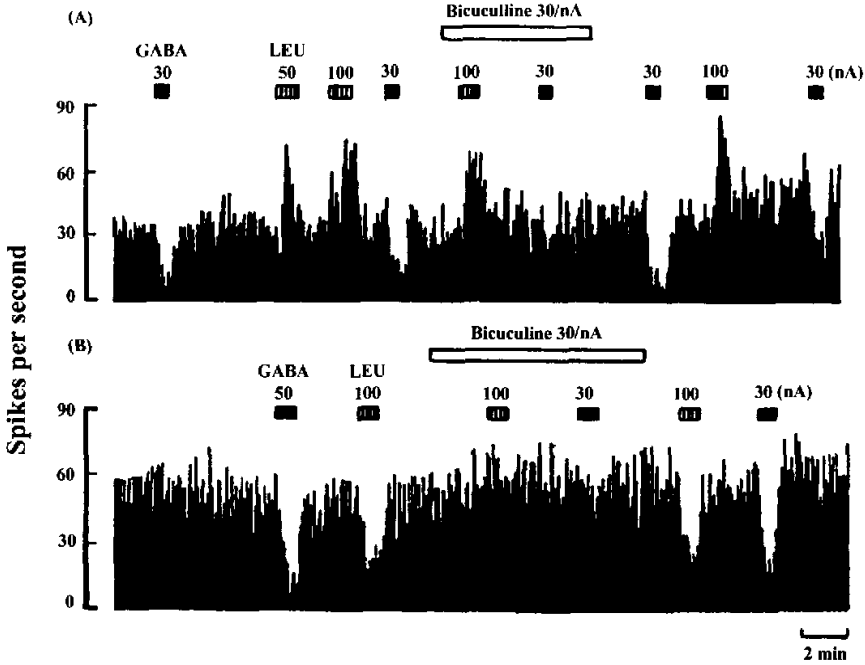

Fig 5. Frequency histogram illustrating the effects of bicuculline, a GABA antagonist, on Purkinje cell responses to leucine-enkephalin (LEU). Bicuculline (30 nA) failed to antagonize leucine-enkephalin (100 nA)-induced excitatory responses (A), but completely antagonized leucine-enkephalin (100 nA)-induced inhibitory responses (B).

阿片样肽类的微离子透入对猫小脑浦肯野氏细胞的作用

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关键词 阿片样肽类; 吗啡; 离子透入法; 浦肯野氏细胞; 小脑

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