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Effects of Opioid Pure Agonists on the Excitibility of Frog Sciatic Nerve Fibers

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Opioid pure agonists, morphine, meperidine and methadone, were used to investigate the effect on the opioid receptor of frog sciatic nerve fibers using sucrose gap apparatus. When applied extracellularly by perfusion, morphine, methadone and meperidine significantly depressed the amplitude of the action potential in frog sciatic nerve fibers as a dose-dependent (10^{-10} M-10^{-2} M) manner. The depression with morphine or methadone was partially antagonized by the simultaneous treatment with a lower (10^{-10} M-10^{-8} M) concentration of naloxone, but that of meperidine was not blocked. When the three opioid agonists were applied intracellularly by placing it in a compartment with a cut end of the sciatic nerve fibers, all of them depressed the amplitude of the action potentials by similar potency, and these reductions significantly blocked by pretreatment of lower concentration (10^{-10} M-10^{-8} M) of naloxone. These results support the previous findings by other workers that the stereospecific opioid receptors of this preparation are located on or near the intracellular opening of the sodium channels which are sensitive to naloxone.

Key words: Opioid pure agonists, Sucrose gap apparatus, Compound action potentials(CAPs), Frog sciatic nerve fibers

INTRODUCTION

Since the existence of multiple opioid receptor subtypes was first proposed by Martin *et al.* (1976), a wide variety of biochemical, electrophysiological, pharmacological and behavioral evidences indicate that opioids exert their effects interacted with μ, δ, k and σ receptors (Zukin, 1982; Smuda and Levie, 1986). As far as the ligand selectivity is concerned for these receptors, it has been observed that morphine and other related classical opioids bind with high affinity to receptors antagonized by naloxone. It is well established that opioids modify the excitability of a variety of neurons in the CNS. Even though the studies on PNS has been conflicting, recent studies have shown that a relatively large concentration (higher than 10^{-4} M) of opioids depress the compound action potentials in the isolated muscle fibers and nerve fibers from different animal models. The purpose of this experiment is to elucidate one of physiological or electrical mechanisms of the opioids in living system and to try to develop one of the detoxication mechanisms of morphinism (acute and chronic) and its abstinence syndrome. We have reported the presence of opioid receptors located on the inner surface of the excitable cell membranes of peripheral nerve fibers from rabbit, guinea pig (Frank and Sudha, 1987) and frog using the sucrose gap technique (Frank and Buttar, 1975). In this paper, we first observed the different effects from the classical pure opioid agonists using two different drug applications.

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MATERIALS AND METHODS

The experiments were carried out on the sciatic nerves isolated from the leopard frog Rana pipines at room temperature (15-18℃). The nerves were desheathed for drug penetration under a dissecting microscope and split longitudinally into two bundles without damage of nerve fibers. In every experiment, we used new frog legs, one bundle for control and the other of bundle for drug from one side leg. For stabilization of split nerves, they were allowed to rest in frog Ringer's solution for 1 hr after desheath. The desheathed nerves were moved to place in a sucrose gap apparatus similar to that modified by Lee and

Frank (1989a and 1990b). The nerve bundle was pulled through each hole of the four rubber membranes in a five-chambered sucrose gap apparatus. The experiments were designed by two different drug applications to compare the opiate effect on the action potentials different action sites of the frog sciatic nerves according to drug applications.

Single sucrose gap experiments

After setting the nerve bundle (about 12 mm) in the bath, the central (the third) chamber was perfused with frog Ringer's solution at the rate of 3 ml/min and the one adjacent chamber (generally the second) was perfused with isotonic sucrose solution (214 mM) at the rate of 2 ml/min.

IsoKCl (123 mM) or drug in isoKCl was applied to the end chamber (left side, the first one) and the other chambers (the fourth and the fifth) were filled with frog Ringer's solution (Frank and Marwaha, 1978, 1979; Frank and Sudha, 1987).

Double sucrose gap experiments

The second and the fourth chambers were perfused with isotonic sucrose solution (214 mM), the central chamber was perfused with frog Ringer's solution, and the other chambers (both ends) were filled with frog Ringer's solution. Drugs in frog Ringer's solution were perfused into the central chamber via a three-way stopcock 1 hr after setting the nerve bundle in the bath.

Electric recording

The compound action potentials were recorded between the two compartments separated by the sucrose gap. Stimulating voltage (the first and the third chamber) and membrane potentials (the third and the fifth chamber) were conducted. The stimulating voltage was set to produce the maximal compound action potentials, and single rectangular pulses of supramaximal strength and 0.01-0.05 m/sec in duration were used for frog sciatic nerves. The experiments were performed using a digital Oscilloscope (Nicolet 4094), and the action potentials were stored in the disk in the disk recorder (XF-44 Nicolet) to analyse the data directly by computer (Hewlett Packard 9816), and to draw the pictures of real action potentials by X-Y Recorder (7015 B, Hewlett Packard).

Solutions and drugs

The composition of the frog Rigner's solution was as follows (in mM): NaCl, 111.87; KCl, 2.47; $CaCl_2$, 1.08; NaH_2PO_4, 0.087; $NaHCO_3$, 2.38; and Dextrose, 11.1. Isotonic sucrose solution contained 214 mM sucrose (Sigma Chem. Co., MA, USA), and isoKCl solution contained 123 mM KCl. The drugs used in this experiment were morphine HCl (May & Baker LTD., Canada), methadone HCl (Winthrop Lab N.Y. USA), meperidine HCl (Winthrop Lab N.Y., USA)) and naloxone HCl (Endo Lab Delaware, USA).

All drugs were dissolved in either isoKCl or in frog Ringer's solution for single sucrose gap experiments or double sucrose gap experiments respectively. All solutions were adjusted at pH 7.1-7.2. Before the treatment of isoKCl or drug, the nerve bundle in the bath

Table 1. Effects of 10^{-4} M opioid agonists on the compound action potentials of frog sciatic nerve by single sucrose gap experiment

Treatment	n	Time in min (% control)						
		0	30	60	120	180	240	recovery
Control	8	100.0	100.5± 1.58	100.4± 1.69	96.7± 1.08	95.6± 1.22	94.0± 1.51	93.6± 2.22
Morphine	7	100.0	98.4± 3.15	96.7± 2.68	92.1± 3.25	84.7± 2.79	78.4± 2.72*	91.6± 4.11
Methadone	6	100.0	98.6± 1.56	93.0± 1.75	87.8± 2.58	80.4± 2.67	73.8± 2.91*	88.6± 2.93
Meperidine	7	100.0	96.9± 2.54	96.6± 1.95	90.5± 1.29	85.2± 2.02	78.2± 2.13*	92.3± 1.21

Mean± S.E., n: numbers of experiments, *P<0.05

Table 2. Effects of 10^{-4} M opioid agonists on the amplitute of the compound action potentials of frog sciatic nerve by double sucrose gap experiment

Treatment	n	Time in min (% control)						
		0	30	60	120	180	240	recovery
Control	7	100.0	100.3± 2.01	99.7± 1.98	99.2± 1.01	97.5± 1.59	95.3± 1.67	94.6± 2.24
Morphine	6	100.0	92.6± 1.96	83.5± 1.79	67.9± 2.58*	59.4± 3.21**	47.2± 2.69***	90.4± 3.51
Methadone	7	100.0	84.8± 1.79	74.6± 2.20	57.3± 5.69**	45.9± 5.20**	37.6± 4.77***	85.2± 2.08
Meperidine	7	100.0	89.7± 1.88	80.4± 1.54	71.6± 3.85	68.0± 3.17*	60.1± 3.69**	93.7± 3.33

Mean± S.E., n: numbers of experiments, *P<0.05, **P<0.01, ***P<0.001

should be rest more than 1 hour for stabilization. The nerve bundle was stimulated and the action potentials were recorded at 2, 5, 10, 20, 30 and 60 min for the first hour and every 30 min thereafter which lasted 4 hr. The means of the responses recorded at each time were compared with each drug condition using student's t test, and $p<0.05$ was taken as the level of significance.

RESULTS

Before testing the effects of opioids on frog sciatic nerve action potentials, control tests lasting 4 hr without drug were conducted using both single sucrose gap method (intracellularly) and double sucrose gap method (extracallulary). In the control experiments, little decrements in the amplitude of compounds action potentials were observed over the 4 hr (about 5%), and we also observed the recovery effects of all drugs in this experiment at 8 hrs.

The control size of the compound action potentials recorded averaged (mean± S.E.) 71.7± 3.0 mV for the double sucrose gap and 68.4± 3.0 mV for the single sucrose gap at the 4 hr (Fig. 3). As the preliminary experiments for various doses (from 10^{-8} M to 10^{-2} M) of three opioid agonists using two applications were performed to determine the proper dose for drug interaction between agonist and antagonist, the doses were chosen 10^{-4} M for agonists (morphine, methadone and meperidine) and 10^{-8} M for antagonist (naloxone).

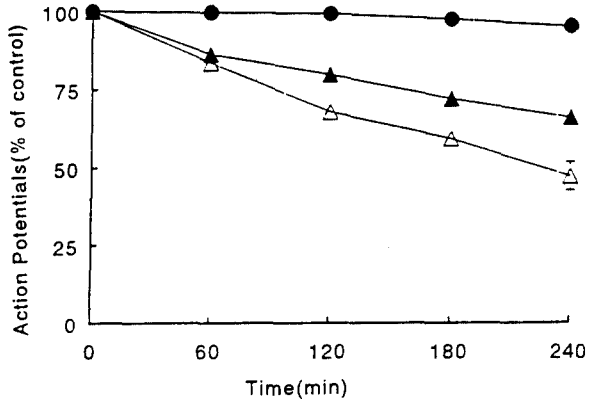

Fig. 1. Effect of morphine (10^{-4} M) with (▲) without (△) naloxone (10^{-8} M) on frog sciatic nerve fibers by double sucrose gap experiment. Closed circles represent control.

Effect of morphine

The effects of morphine using two different applications were shown in Table 1 and 2. and Fig 1. Extracellularly applied (double sucrose gap technique) morphine depressed significantly the compound action potentials of frog sciatic nerve around 50% (Table 2 and Fig. 1), but only 20% depression by morphine was observed in single sucrose gap experiment at 4 hrs (Table 1). Naloxone blocked the depressant effect of morphine on the compound action potentials of frog sciatic nerve fibers in intracellular application (Table 3), but only partially blocked in extracellular application (Fig. 1).

Effect of methadone

The action of methadone on the compound action potentials of frog sciatic nerve fiber were shown in Table 1 by intracellular treatment and in Table 2 and Fig. 2 by extracellular application. In single sucrose gap experiment, methadone depressed only around 20% of the compound action potentials of frog sciatic nerve fibers (Table 1), but this drug significantly depressed the compound action potentials in double sucrose gap experiment (about 60% decrement)(Table 2 and Fig. 2). Naloxone blocked the depressant effect of methadone in intracellular experiment (Table 3) but did not fully block the depression in extracellular application (Fig. 2).

Effect of meperidine

The effect of meperidine in two different application was shown in Table 1 and 2 and Fig. 3. In single sucrose gap experiment, the effect of this drug on the compound action potentials of frog sciatic nerve fivers was similar to that of morphine (Table 1), and it signifi-

Table 3. Effects of 10^{-8} M Naloxone on the depressant actions of the opioid agonists in frog sciatic nerve fibers by single sucrose gap experiments

Treatment	n	Time in min (% control)						
		0	30	60	120	180	240	recovery
Control	5	100.0	100.3± 1.06	100.2± 3.11	98.3± 1.21	96.3± 2.21	96.2± 1.98	94.3± 3.21
Naloxone only	8	100.0	99.3± 0.84	98.7± 1.73	92.5± 2.77	87.9± 2.80	89.1± 2.29	91.3± 2.22
+Morphine	6	100.0	98.3± 1.21	96.3± 2.15	97.2± 2.35	94.3± 3.12	92.3± 1.35*	92.3± 2.54
+Methadone	5	100.0	98.2± 0.88	97.4± 1.96	96.9± 1.90	93.8± 2.93	93.2± 2.29*	91.8± 3.51
+Meperidine	4	100.0	100.2± 1.53	96.7± 1.38	98.2± 2.35	96.4± 1.69	94.3± 2.98*	93.2± 1.63

Mean± S.E., n: numbers of experiments, These values were compared with the values in Table 1.

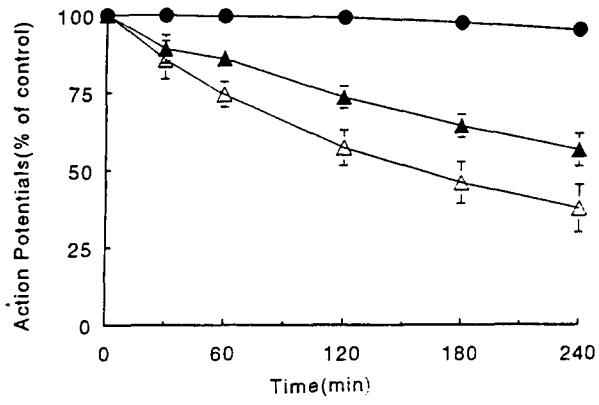

Fig. 2. Effect of methadone (10^{-4} M) with (▲) or without (△) naloxone (10^{-8} M) on frog sciatic nerve fibers by double sucrose gap experiment. Closed circles represent control.

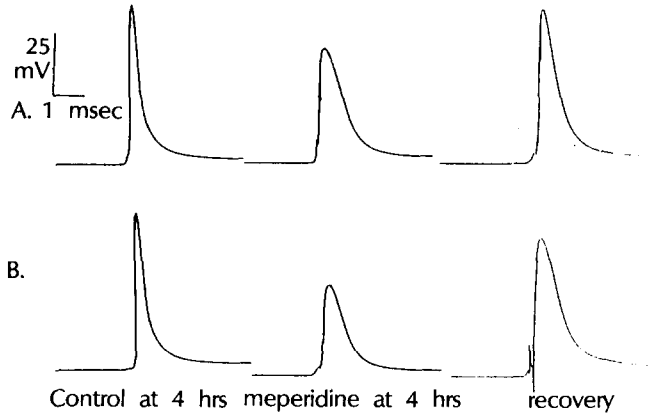

Fig. 3. Effects of 10^{-4} M of meperidine applied both intracellularly (single sucrose gap experiment) and extracellularly (double sucrose gap) on action potentials of a frog sciatic nerve fibers.
A: intracellularly B: extracellularly

cantly depressed the compound action potentials of frog sciatic nerve fibers in extracellularly applied experimet (Table 2). The effect of meperidine was not so potent as those of morphine and methadone in double sucrose gap experiment. Naloxone blocked the depressant action of meperidine in intracellular treatment experiment (Table 3) but it did not fully blocked the depression in extracellular application experiment.

Effect of naloxone

Because naloxone shows the biphasic pattern depending on using opioid agonists in peripheral tissue (skeletal muscles or nerves)(Lee and Frank, 1989a, 1989 b), we should find the antagonistic dose of naloxone for this preparation from preliminary experiment. 10^{-8} M of Naloxone blocked significantly the depressant actons of pure agonists in intracellular application experiment (Table 3), but only partially blocked depression in extracellular treatment experiment (Fig. 1 and Fig. 2).

DISCUSSION

The results obtained in the present study show that opioid agonists depress the excitable cell membrane of frog sciatic nerve both in single and in double sucrose gap experiment. As naloxone significantly blocked the depressant effects of opioid agonists in single sucrose gap experiment but not in double sucrose gap experiment, we could assume that the opioid agonists might work their actions by two different ways, that is, one of which is stereospecific opioid receptor being sensitive to naloxone but the other is not.

Because opioid receptor-related drugs (pure agonist, partial agonist, agonist-antagonist, antagonist etc.) show the various responses depending on the different tissues or different drug applications (Hunter and Frank, 1979; France *et al.*, 1984; Shefner *et al.*, 1981), the distinction between a heterogeneous cooperative receptor sites and a homogenous multisubsite receptor on the basis of equilibrium binding and activity curves is experimentally very difficult, and both models also share biphasic observable properties. With theoretical aspects of drug-receptor interactions reviewed by Ariens *et al.* (1957), the pharmacological characteristics for dual actions of narcotics cannot be explained on the basis of a single homogenous receptor population. Because that theory supported the existence of two functionally different opioid receptor-related agonists on the excitable membrane, we decided to use two different drug application method using sucrose gap technique as our articles previously described. In general, the results from electrophysiological studies for the action of opioids on neurons both in the CNS and in the PNS in intact animal are difficult to interpret the pattern of mechanisms, because there are several affecting factors, that is, the uncertainty of the primary site of drug action, the ignorance of tissue concentrations and the complication of anesthesia.

Therefore, we have sought to avoid some of these difficulties by studying the effects of opioids on the isolated peripheral tissues which can be maintained in vitro studies. In our present experiment, the differences of results between in intracellular application and in extracellular application means that these opioids may have two different sites in this preparation.

In single sucrose gap experiments, drugs were allowed to diffuse through the axoplasm of the axons to reach their own site of action on the other side of the sucrose gap, differing from double sucrose gap experiments which drugs were allowed to cross the membrane to get the site action of receptor. Drug

receptor is the site at which a drug unites to produce its effect in the living system (Frank, 1968), therefore, if the opioid drug exerts the effect on excitable membranes it means that there is an opioid receptor on these membranes (Hodgkin and Katz, 1949; Hu and Rubly, 1983).

Our study showed the complete antagonism of simultaneous treatment of naloxone to the depressant effects of opioid agonists in single sucrose gap experiment. These results may suggest the stereospecific opioid receptor cited on inner membrane of this preparations which is sensitine to naloxone. Considerable evidence has been presented that the presence of stereospecific opoid receptors on excitable cell membranes (Ary and Frank, 1983; Frank, 1985) which depress the compound action potential amplitudes when activated by opioid agonist in frog skeletal muscle (Frank and Buttar, 1975), in frog sciatic nerve, in squid giant axon (Frazier *et al.*, 1972), and in mammalian nerve fiber (Jurna and Grossman, 1977; Frank and Sudha, 1987).

But our results in double sucrose gap experiment showed the lack of complete antagonism of pretreated naloxone to the depressant effects of perfused opioid agonists, it means that another non-specific opioid receptor may be cited on the surface membrane of this preparations which is not sensitive to naloxone. It has been reported that the effects of pure agonists in PNS show in two different manners (Hunter and Frank, 1979) being quite different feature of buprenorphine (Lee and Frank, 1989a).

It has been also suggested that opioid agonists produce both a nonspecific local anesthetic-like depression of excitability and a stereospecific depression of sodium conductance (gNa)(Frank, 1975, 1985). The two ways are follows; one of which is mediated through stereospecific receptor (Seeman *et al.*, 1972) which is on or near the inner surface of membrane being sensitive to sodium channel (McFadzean, 1988; Smuda and Levie, 1986), and the other is mediated not stereospecific receptor (Yuge *et al.*, 1985) on outer surface of membrane which is insensitive to antagonist but only showing a local anesthetic-like effect.

The results presented in this study suggest the presence of stereospecific opioid receptor located on the intracellular surface of the peripheral axon which is probably closely associated with or on the sodium channel and also sensitive to naloxone. This result may support a possible physiological role for this stereospecific opioid receptor as a site of action for partial agonist like buprenorphine. But pure agonists may also interact the other non-streospecific opioid receptor on frog sciatic nerve fibers.

Additional experiments should be required to determine whether this finding has generality to other synthetic opioid agonists.

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