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Agonist-Antagonist Effects of Buprenorphine on Action Potentials of Frog Sciatic Nerve Fibers

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ABSTRACT

Buprenorphine, one of the mixed agonist-antagonist opioid drugs was used to inverstigate the opioid receptor on frog sciatic nerve A fibers.

Action potentials were recorded for 4 hrs by a sucrose gap apparatus which were separated by four rubber membranes.

To examine the one of the mechanism of action of buprenorphine, meperidine or naloxone was added after or before the treatment of buprenorphine.

The results of this experiment were as follows:

1. Buprenorphine suppressed significantly the compound action potentials of frog sciatic nerve, and the maximal effects were shown both at 10^{-4} M and at 10^{-8} M.

2. The dose-response relationship of buprenorphine on the depressant effect in frog sciatic nerve was biphasic and inverted U-shaped.

3. Buprenorphine blocked the effect of Meperidine (10^{-3} M) on this preparation.

4. The depressant effect of Buprenorphine on frog sciatic nerve was blocked by 10^{-8} M naloxone.

From the above results, buprenorphine acts as one of agoinist-antagonistic effect on frog sciatic nerve, and the opioid receptor on this preparation is located on or near the intracellular opening of the sodium channels, which are sensitive to naloxone.

Key Words: Buprenorphine, Mixed agonist-antagonist, Frog sciatic nerve A fiber, Compound action potentials, Meperidine, Naloxone

INTRODUCTION

In the search for alternative analgesics to morphine, studies have been continued to focus on those compounds that possess both antinociceptive and narcotic antagonist properties.

Buprenorphine, a highly lipophilic synthetic dervatives of thebaine-oripavines, has been classified as a partial agonist at receptors (Martin, 1979) with mixed agonist-antagonist analgesic properties (Cowan *et al.*, 1977b; Rance, 1979). Buprenorphine has a rapid onset and a long duration of action, and its behavioral characteristics are similar to those of both morphine and naloxone (France *et al.*, 1984).

In recent years, a number of studies concerned with opioid drugs and opioid receptors have been increased in physiological processes, It also has been demonstrated that the opioids modify the excitability of various neurons both in CNS and in PNS.

Considerable evidence has shown that opioid durgs, in a relatively large concentration, depress the action potentials of the isolated frog sciatic nerves (Hunter and Frand, 1979) and skeletal muscle (Frank and Buttar, 1975; Frand and Marwaha, 1978) suggesting the presence of stereospecific opiod receptors on peripheral excitable cells (Frand; 1985; Ary and Fran, 1983).

The purpose of the present study was to investigate the effect of buprenorphine on action potentials of the frog sciatic nerve as one of mixed agonist-antagonist analgesic and also to elucidate one of the mechanisms of action of this drug.

MATERIALS AND METHODS

The experiments were carried out on the isolated sciatic nerves from the leopard frog Rana pipines at room temperature (15-18° C). The

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nerves were desheathed under a dissecting microscope and split longitudinally into two bundles. For stabilization of split nerves, they were allowed to rest in frog Ringer's solution for 1 hr. The desheathed nerves were moved to place in a sucrose gap apparatus similar to that described by Kosterlitz and Wallis (1964) and as modified by Frank and Hunter (1979), Frank and Sudha (1987).

The nerve bundle was pulled through each hole of the four rubber membranes in a five-chambered sucrose gap apparatus. The experiments were designed two different ways to compare the effect of drugs on the action potentials of the frog sciatic nerves for drug conditions.

Single sucrose gap experiments

After setting the nerve bundle in the bath, the central (the third) chamber was perfused with free 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 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 chamber (both ends) was 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 action potentials in this experiment were recorded between the two compartments separated by the sucrose gap. Stimulating voltage (the first and the third chamber) and membrane portntials (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 supramaxial strength and 0. 01-0.05 msec 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 Nicoletr) to analyse the data directly by computer (Hewlett Packard 9816), and to draw the pictures of real action potentials by X-Y Recorder (7015B, Hewlett Packard).

Solutions and drugs

The composition of the frog Ringer'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, and IsoKCl solution contained 123 mM KCl. The drugs used in this experiment were buprenorphine HCl (Reckitt and Coleman Ltd., Kingston-upon-Hull), Naloxone HCl (Endo Lab.), and meperidine HCl (Winthrop Lab.)

All drugs were dissolved in either IsoKCl or in frog Ringer's solution for single sucrose gap experiments and double sucrose gap experiments, respectively.

All solutions were adjusted pH at 7.1-7.2.

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

Preliminary experiments for various doses for three drugs were designed to find the proper dose for this experiment.

For all the different nerve, control tests were carried out without any application of drug lasting for more than 1 hr.

The drug were applied to a cut end of the nerve in the left chamber by single sucrose gap experiment, and central perfusion of drugs were carried out by double sucrose gap experiment together, sucrose perfusions were carried out via 2nd and fourth chamber at the rate of 2 ml/min during experiment. Buprenorphine was tested in concentrations from 10^{-10} M to 10^{-2} M on these preparations (Fig 1.), and two concentrations (10^{-4} M & 10^{-8} M) of buprenorphine were chosen to elucidate the mechanism of action of drug related to the

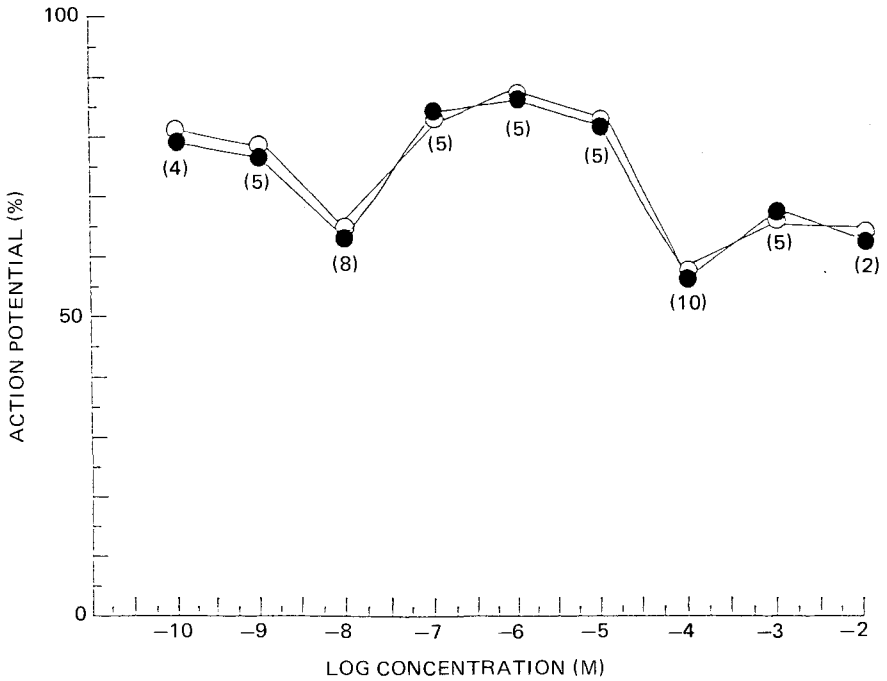

Fig. 1. Dose-Response of Buprenorphine by single (●) or doulbe (○) sucrose gap experiment at 4 hr.

Table Effects of buprenorphine on the compouind action potentials of frog sciatic nerve fibers with or without naloxone by single sucrose gap experiment

Treatments	n	Time in mine			
		60	120	180	240
Buprenorphne 10^{-8} M only	8	92.7±1.69	80.1±3.18	70.7±4.20	63.0±4.27
with naloxone 10^{-8} M	6	98.0±2.92	93.4±2.25*	90.2±2.63**	83.5±1.28**
Buprenorphine 10^{-4} M only	6	92.0±1.94	76.4±3.15	65.6±2.44	59.1±2.20
with naloxone 10^{-8} M	7	92.7±1.58	87.9±3.21*	79.2±3.71**	77.7±2.89**

Mean±S.E. n; Numbers of Experiments *$p<0.05$ **$p<0.01$

opiate receptor. Also meperidine was tested from 10^{-8} M to 10^{-3} M, and 10^{-3} M was chosen to observe the drug-interaction with buprenorphine on this preparation. Similarly, various concentrations of naloxone ($10^{-10}-10^{-2}$ M) were tested on the frog sciatic nerve, and 10^{-8} M was chosen to examine the drug-drug interaction with buprenorphine or meperidine in this experiment.

Effects of buprenorphine

All the concentrations between 10^{-10} M and 10^{-2} M of buprenorphine were observed the effect on the action potential of the frog sciatic nerve both by single sucrose gap technique and by double sucrose gap technique.

The dose-response curves of buprenorphine on this preparation were inverted U-shaped in both sucrose gap experiments and two most effective concentrations of buprenorphine on the action potential were shown at the concentration of 10^{-8} M and 10^{-4} M (Fig. 1 and Table).

The typical trace of action potential by 10^{-4} M of buprenorhine was shown is Fig. 2 and this

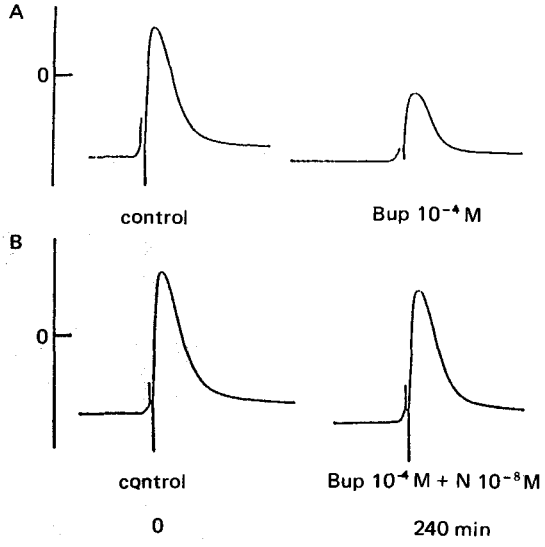

Fig. 2. Effect of buprenorphine applied by single sucrose gap experiment with or without naloxone. In A and B are two seperate experiments using each bundles of nerve fibers split from a single sciatic nerve. Duration of drug exposure is listed on the line below the records.

action was blocked by 10^{-8} M of Naloxone. (Fig. 2 and Table).

Effects of meperidine

When meperidine was added to the frog sciatic nerve using single sucrose gap technique to apply intracellularly, it did not show any significant depression of the action potential. In contrast, meperidine 10^{-3} M was perfused with central chamber to apply extracellularly to this preparation for 4 hr, the rate of decrease of the amplitude of the compound action potential was about 60% of control (Fig. 3). The depressant effect of meperidine 10^{-3} M on the action potential of the frog sciatic nerve was antagonized by naloxone 10^{-8} M in this study (Fig. 3 and 4).

Effect of Buprenorphine on Meperidine

When buprenorphine (10^{-8} M) with meperidine (10^{-3} M) were perfused via central chamber to apply extracellularly to the frog sciatic nerve, the depressant effect of meperidine (10^{-3} M) was blocked by buprenorphine (10^{-8} M) as naloxone (10^{-8} M) did (Fig.4).

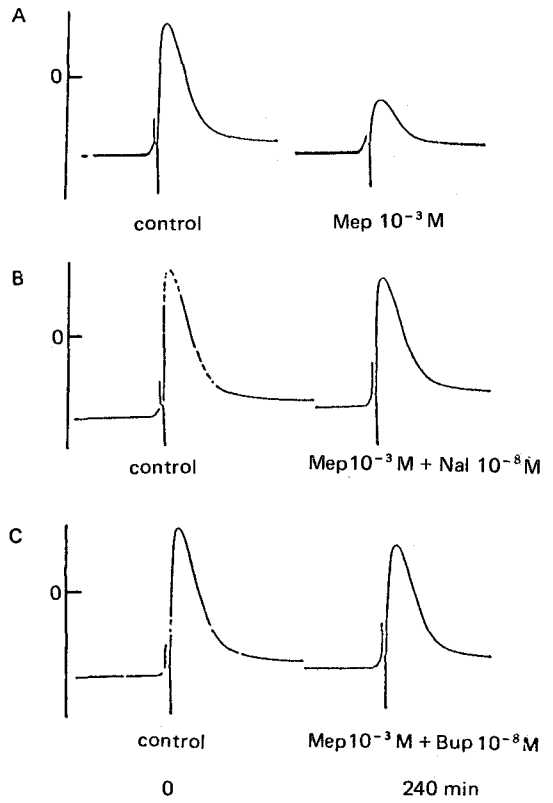

Fig. 3. Effect of meperidine applied by double sucrose gap experiment with or withour naloxone or buprenorphine. In B and C are two seperated experiments using each bundles of nerve filbers split from a single sciatic nerve. Duration of drug exposure is listed on the line below the records.

And also buprenorphine (10^{-4} M) blocked the depressant effect of meperidine (10^{-3} M) the compound action potential of the frog sciatic nerve as 10^{-8} M of buprenorphine did (Fig. 4).

DISCUSSION

On the basis of intrinsic pharmacological activity, opioids can be classified as pure agonist, partial agonist and antagonists. Partial agonists may act as agonist or antagonist depending upon the pharmacological test and the degree of receptor activation that is required to elicit agonistic or antagonistic action. In general, the mixed agonist-antagonist opioids are a heterogenous group of

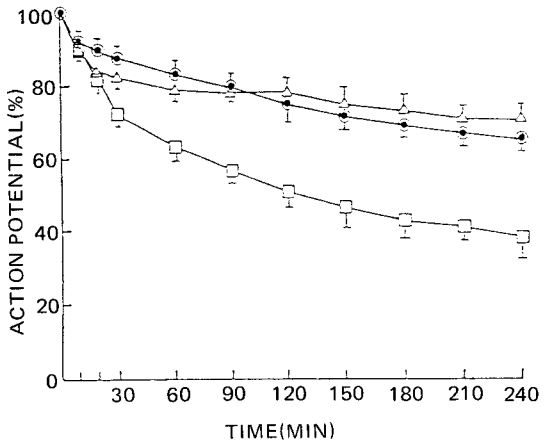

Fig. 4. Effects of meperidien with buprenorphine or naloxone.
□ meperidine 10^{-3} M only (n=6)
◉ meperindine with buprenorhine 10^{-4} M (n=5)
▽ meperindine with naloxone 10^{-8} M (n=5)

compounds that can be separated into several subsets on the basis of differences in their agonistic action. Buprenorphine,a member of the semisynthetic thebaine-oripavine derivatives is structurally close to an analogue of the potent opioid agonist etorphine, and also the antagonist diprenorphine. It has been classified by Martin *et al.,* (1976) as a morphine-like (μ) partial agonist with respect to analgesia, and many dose-response curves in various animal test situations demonstrate a plateau at less than maximal effects as might be expected for a partial action (Cowan *et al.,* 1977a). Buprenorphine is approved for sale in 45 countries (Lewis, 1985). Its main advantage over morphine is that the dose need not be increased during chronic administration in animal study (Cowan *et al.,* 1977a; rance 1979) and in humans (Jasinski *et al.,* 1978), but it induces dependence like other opioids even though few signs of withdralwal are seen in direct dependence because of reflection of the slow dissociation of the drug from the binding site (Dum *et al.,* 1981; McCarthy and Howlett 1984). Chang *et al.* (1981) reported that buprenorphine is relatively nonselective opioid for the opioid receptor subtype since the affinity for μ, δ and k receptors has not been accurately determined. Since the mixed groups of agonist-antagonist opioids have frequently been shown to produce inverted U-shaped dose-response curves in several different kinds of experiments, it has been hypothesized that this effect is one of the characteristics of the mixed type opioids. Because opioid drugs show the varying responses depending on the different tissues or different drug conditions, the distinction between heterogeneous cooperative receptor sites and a homogenous multisubsite receptor on the basis equilibrium binding and activity curves is experimentally difficult and both models share most of their observable properties.

There are several reports that the action potential could be blocked more easily by intracellular, compared to extracellular application of opioids in squid giant axon (Frazier *et al.,* 1972 and 1973) so we decided to use both aplications using the sucrose gap technique.

To study one on the mechanism of action of buprenorphine as a mixed agonist-antagonist opioid drug, it was observed that the effect of buprenorphine on the action potential of the frog sciatic nerve using sucrose gap techniques.

In gerenal, electrophysiological studies of the action of opioids on neurons both in the CNS and in the PNS in intact animal are often difficult to interpret due to 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 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. but in double sucrose gap experiments, drugs were allowed to cross the membrane to get the site action of receptor. The results from this experiment showed that buprenorphine depressed the amplitude of the compound action potential of the frog sciatic nerve, and it produced the biphasic dose-response curve showed two peak points both in the concentration of 10^{-8} M at lower dose level and in the concentration of 10^{-4} M at higher dose level. Buprenorphine showed to display such a bell-shaped dose response curve, and this cannot be accounted for within the context of the receptor theory for partial agonist.

Currently, there are two types of molecular hypotheses that can account for the bell-shaped dose-response curves.

The first hypothesis assumes the existence of two interdependent receptor sites termed non-

competitive auto inhibition (Ariens *et al.*, 1964; Cowan *et al.*, 1977b) and the second one assumes the existence of only one type of receptor site; bell-shaped dose-response curves may be generated either by multiple subsites of drug attachment within the same receptor (DeLeon *et al.*, 1979). With theoretical aspects of drug-receptor interactions reviewed by Ariens *et al.*, (1964), the pharmacology of the narcotics with dual action cannot be explained on the basis of a single homogenous receptor population (Rance *et al.*, 1979), and they supported the existence of two functionally related opioid receptor populations. And Sadee *et al.*, (1982) reported buprenorphine showed the bell-shaped dose response curve which noncompetitive auto inhibition occurred among the opioid receptor subtypes.

Drug receptor is the site at which a drug unites to produce its effect in the living system, therefore if the opioid drug exerts the effect on excitable membranes it means that there is an opioid receptor on these membranes.

Considerable evidence has been presented that the presence of stereospecific opioid receptors on excitable cell membranes which depress the action potential production when activated by opioid agonist in frog skeletal muscle (Frank, 1975), in frog sciatic nerve (Frnk and Hunter 1979) and in mammalian nerve fibre (Jurna and Grossman 1979).

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). For elucidating the effect of buprenorphine on the action potential of the frog sciatic nerve, it was observed that drug interactions of buprenorphine with meperidine or naloxone were examined using both single sucrose gap experiments and double sucrose experiments.

In this experiment, buprenorphine blocked the depressant effect of high dose of meperidine (10^{-3} M) on the action potential of frog sciatic nerve as naloxone did.

Since buprenorphine is highly lipophilic than meperidine, it is more quickly transferred to the action site through the membrane and to get more easily to receptor when perfused together by double sucrose gap experiment.

Because buprenorphine is highly lipophilic, longer action and about 25-50 times more potent than morphine (Bryant *et al.*, 1983) and also buprenorphine possesses low physical dependence liability due to slow dissociation from receptor binding site, it is assumed that buprenorphine can easily occupy the stereospecific receptor which exerts the real opioid drug effect, to antagonize the effect of meperidine. When high dose of meperidine is added to this preparation by intracellular application, it did not show the depressant effect like that of buprenorphine.

The results obtained from his experiment suggest that buprenorphine depressed the amplitude of the compound action potential of the frog sciatic nerve fibres and this effect was antagonized by low concentration of naloxone, therefore this depression effect was mediated through stereospecific opioid receptor unlike that of meperidine.

It seems clear that the effect of intermediate intrinsic activity of buprenorphine into a single receptor system interacting with a pure agonist will be antagonistic effect if the concentration of pure agonist is high.

The effect of meperidine of this preparation is due to two mechanisms but that of buprenorphine is mediated through stereo-specific receptor which is on or near the inner surface of membrane sensitive to sodium channel.

The results presented in this study suggest the presence of stereo-specific opioid receptor located on the intracellular surface of the peripheral neuron axon, and this receptor is probably closely associated with or on the sodium channel.

This may suggest a possible physiological role for this stereo-specific opioid receptor as a site of action for partial agonist.

Additional experiments are required to determine whether this finding has generality to other agonist and different kinds of tissues, particularly those which differ from meperidine in affinity and selectivity for receptor subsites.

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= 국문초록 =

개구리 좌골신경에 대한 Buprenorphine의 작용 양상

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함께 투여되는 마약성진통제에 따라 효능제 또는 길항제(mixed agonist-antagonist)로 작용하는 buprenorphine을 사용하여 개구리 좌골신경에 존재하는 아편수용체의 성질을 검토하였다.

실험은 sucrose-gap apparatus를 사용하여 활동전압에 대한 영향을 관찰하였으며 약물의 상호작용을 검토하고자 meperidine 또는 naloxone을 사용하여 다음과 같은 결과를 얻었다.

1) Buprenorphine은 현저하게 개구리 좌골신경의 활동전압을 저하시켰다.

2) 두 개의 최고작용농도를 보였는데 저농도에서는 10^{-8} M, 또 고농도에서는 10^{-4} M에서 현저하게 작용을 나타내었다.

3) Buprenorphine은 meperidine의 활동전압 저하작용을 의의있게 억제하였다.

4) Naloxone에 의하여 Buprenorphine의 저하 작용이 억제되었다.

위의 실험결과로 Buprenorphine은 순수 마약성진통제의 작용을 억제하여 차단제로 작용하는 한편 또한 효능제로 활동전압을 의의있게 억제하였다. 이로써 개구리 좌골신경에 있는 아편수용체는 세포막에 또는 세포막 주위에서 Na^+−Channel이 활성화되는 데 중요한 역할을 하는 것으로 간주되며 이 수용체는 또한 Naloxone에 매우 민감한 반응을 나타낸다.