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Inhibition of noradrenaline release from cerebrocortical synaptosomes and stimulation of synaptosomal Na^+, K^+-ATPase activity by morphine in rats

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Abstract—The effects of morphine on noradrenaline (NA) release from rat cerebrocortical synaptosomes and on the synaptosomal Na^+,K^+-ATPase activity were determined. Morphine (10^{-3}–10^{-5} M) caused a dose-related inhibition of enhanced prelabelled [^{3}H]NA release evoked by a high concentration of K^+ from synaptosomes and this inhibitory action of morphine was antagonized by the specific antagonist naloxone (10^{-4}, 10^{-5} M). Morphine dose-dependently stimulated the synaptosomal Na^+,K^+-ATPase activity but not Ca^{2+}-ATPase activity in the incubation medium containing $2{\cdot}2 \times 10^{-6}$–$4{\cdot}7 \times 10^{-7}$ M free Ca^{2+}, and this stimulatory effect was antagonized by naloxone. These results suggest that morphine may have some role in the suppression of membrane depolarization and/or the release of NA through its stimulatory action on the Na^+,K^+-ATPase activity in rat cerebral cortex.

Morphine and opiate analgesic drugs exert their effects on the central nervous system by interacting with receptors located on neuronal membranes and depress the release of neurotransmitters resulting from the inhibition of depolarization of the individual neurons (Cardenas & Ross 1976; Taube et al 1976; Cerreta et al 1977; Subramanian et al 1977; Aghajanian 1978; Jhamandas & Sutak 1980). However, the biochemical mechanism of the inhibitory or depressant action on neurotransmitter release has not been thoroughly elucidated.

Membrane Na^+,K^+-ATPase (EC 3.6.1.3) has been shown to be involved in active ion transport across the cell membrane and considered to be the biochemical basis of the sodium pumping function (Skou 1965; Schwartz et al 1975). The Na^+,K^+-ATPase was shown to have a regulatory role in the release of neurotransmitters or other intracellular substances (Vizi 1977; Meyer & Cooper 1981; Nishikawa et al 1985). Desaiah & Ho (1977) showed that the Na^+,K^+-ATPase activity in the crude nerve ending fraction prepared from mice which had been implanted with morphine pellets was increased compared with control. Hàjek et al (1985a) reported that morphine and methionine-enkephalin enhanced the activity of the membrane Na-K pump in frog spinal cord. On the other hand, several investigators detected no stimulatory effect of morphine on the membrane Na^+,K^+-ATPase activity in-vitro (Desaiah & Ho 1977, 1979; Ventura et al 1987). Because of the above inconsistency, we examined the possible relationship between the effects of morphine on Na^+,K^+-ATPase activity, transmitter release and membrane depolarization. Here we describe the significant stimulatory action of morphine on synaptic membrane Na^+,K^+-ATPase activity from rat cerebral cortices in the incubation medium containing $2{\cdot}2 \times 10^{-6}$ to $4{\cdot}7 \times 10^{-7}$ M free Ca^{2+}. Our finding may contribute to the understanding of one of the mechanisms by which morphine inhibits the neurotransmitter release from the central nervous system.

Materials and methods

Preparation of synaptosomes. Male Wistar rats, 200–300 g, were killed by exsanguination after a blow on the head. The cerebral cortex was rapidly removed and homogenized in 0·32 M sucrose containing 3 mM ethylendiaminetetraacetate (EDTA). The synaptosomal fraction was isolated by a slightly modified method of Barker et al (1972). The homogenates (10% w/v) were centrifuged for 15 min at 1000 *g* and the supernatant was centrifuged for 20 min at 13 500 *g* to sediment the crude mitochondrial fraction. This fraction was gently resuspended by hand in a glass-Teflon homogenizer, applied onto a discontinuous gradient of 1·2 and 0·8 M sucrose each containing 3 mM EDTA, and then centrifuged for 90 min at 98 000 *g* in the swing-out rotor (RPS50-2-151; 6 × 5 mL) of a Hitachi 55P-7 ultracentrifuge. The materials from the interphase between 1·2 and 0·8 M sucrose were collected by suction with a syringe, diluted about ten times with 0·32 M sucrose containing 3 mM EDTA, and sedimented by centrifugation for 20 min at 16000 *g*. The synaptosomal pellets were resuspended in 0·32 M sucrose and used for subsequent experiments.

Assay for [^{3}H]NA release. Preparation of [^{3}H]NA-containing rat brain synaptosomes and measurement of [^{3}H]NA release were carried out as described before (De Langen et al 1979).

Assay for Na^+,K^+-ATPase activity and Ca^{2+}-ATPase activity. Synaptosomal pellets were gently homogenized with distilled water and used as an enzyme source. About 100 μg mL^{-1} (final) of synaptosomal membrane protein was used in each assay. Details of the media and conditions for Na^+,K^+-ATPase (Nishikawa et al 1988, 1989) and Ca^{2+}-ATPase (Ross & Cardenas 1983) activities have been reported previously. The amounts of protein (Lowry et al 1951) and inorganic phosphate (Taussky & Shorr 1953) in the supernatant were measured by previously established methods.

Chemicals. The chemicals used were: 1-[7-^{3}H]-NA (20 Ci $mmol^{-1}$, New England Nuclear), (−)-NA hydrochloride (Sigma), morphine hydrochloride (Takeda Chem. Ind. Ltd.), naloxone hydrochloride (Sigma), desipramine hydrochloride (Sigma), disodium ATP (Sigma, prepared by phosphorylation of adenosine, grade I) and ouabain (Sigma).

Statistical analysis. Statistical analysis was performed using the independent *t*-test for comparison. The results in the text and figures are expressed as the means ± s.e.m.

Results

Inhibition by morphine of [^{3}H]NA release evoked with high K^+ from synaptosomes and antagonism by naloxone. To ascertain whether morphine inhibits the prelabelled [^{3}H]NA release evoked by a high concentration of K^+, its effect was tested using cerebrocortical synaptosomes obtained as described above. Fig. 1 shows the effect of morphine and the antagonistic effect of naloxone on [^{3}H]NA release evoked by a high concentration of K^+ from synaptosomes of cerebral cortices. Morphine (10^{-3}–10^{-5} M) dose-dependently inhibited the K^+-stimulated release of [^{3}H]NA, and the specific antagonist naloxone (10^{-4}, 10^{-5} M) antagonized the inhibitory effect of morphine.

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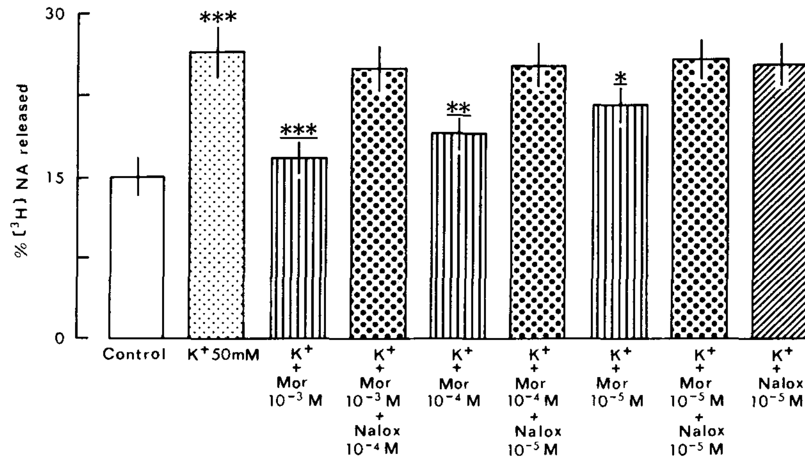

FIG. 1. Effect of morphine on [^{3}H]NA release from cerebrocortical synaptosomes evoked by high K^+ concentration. [^{3}H]NA-containing synaptosomes (about 300 μg synaptosomal protein) were superfused with medium containing 1·2 mM $CaCl_2$. Morphine and naloxone were added to the medium 10 min before the application of high K^+ (50 mM). After 2 min (stimulation period), the synaptosomes were isolated and counted for radioactivity. ^{3}H overflow during 2 min collection is expressed as a percentage of the total radioactivity in the synaptosomes at the start of stimulation. Columns represent mean values for 5 to 7 determinations with s.e.m. as a vertical line. *** Significantly different ($P<0·001$) from control. ** Significantly different ($P<0·01$) from the response caused by 50 mM K^+ alone, ** ($P<0·01$). Mor: morphine, Nalox: naloxone.

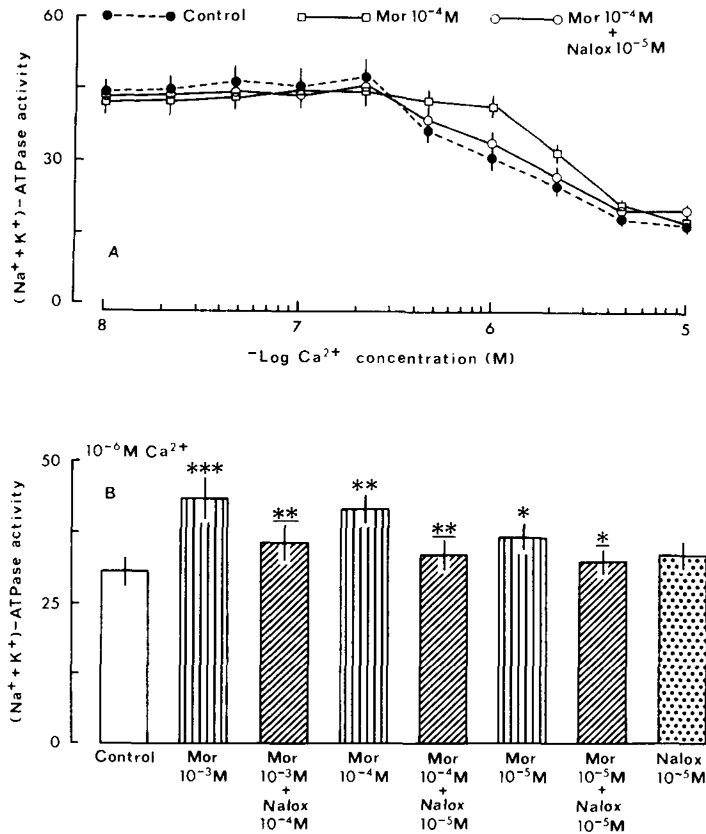

FIG. 2. A. Effect of morphine on synaptosomal membrane Na^+,K^+-ATPase activity from the cerebral cortices in the medium containing various concentrations of free Ca^{2+}. Synaptosomal membranes were incubated with morphine for 10 min before the addition of ATP. The Na^+,K^+-ATPase activities were expressed as μmol inorganic phosphate formed (mg membrane protein)$^{-1}$ h^{-1}. The total (free and bound) calcium concentration of the incubation medium (100 μg mL^{-1} H_2O synaptosomal membrane protein) was $6·35 \pm 0·03 \times 10^{-7}$ M. The concentrations of free Ca^{2+} were adjusted with Ca^{2+}-EGTA buffer and calculated as described by Ogawa (1968). Each point represents the mean of 7 to 10 determinations with the bar denoting s.e.m. B. Effect of morphine on synaptosomal membrane Na^+,K^+-ATPase activity in the medium containing 10^{-6} M free Ca^{2+}. *** Significantly different ($P<0·001$) from control, **($P<0·01$), *($P<0·05$). ** Significantly different ($P<0·01$) from the corresponding responses caused by morphine alone, *($P<0·05$). For abbreviations, see Fig. 1.

Stimulation by morphine of synaptosomal Na^+,K^+-ATPase activity and antagonization by naloxone. Morphine (10^{-4} M) was tested for its ability to influence the Na^+,K^+-ATPase and Ca^{2+}-ATPase activity of cerebrocortical synaptic membranes in the medium containing various concentrations of free Ca^{2+}. As is evident from Fig. 2A, morphine (10^{-4} M) increased the synaptosomal Na^+,K^+-ATPase activity but not Ca^{2+}-ATPase activity (data not shown) in the medium of $2{\cdot}2 \times 10^{-6}$–$4{\cdot}7 \times 10^{-7}$ M free Ca^{2+}. The stimulatory effect was dose-dependent and antagonized by naloxone (10^{-4}, 10^{-5} M) in the medium containing 10^{-6} M free Ca^{2+} (Fig. 2B).

Discussion

Our results showed that morphine inhibited the enhanced release of [^{3}H]NA evoked by high K^+ from synaptosomes and this inhibitory action was antagonized by naloxone. Also, morphine dose-dependently increased the synaptosomal Na^+,K^+-ATPase activity of rat cerebral cortex in the incubation medium containing $2{\cdot}2 \times 10^{-6}$–$4{\cdot}7 \times 10^{-7}$ M free Ca^{2+} and naloxone antagonized the stimulatory effect of morphine. Our findings concerning NA release confirm the earlier reports of Montel et al (1974) and Göthert et al (1979) describing the inhibition by morphine or methionine- enkephalin of [^{3}H]NA release evoked by high K^+ from slices of rat cerebral cortex.

It is well accepted that depolarization due to the increased intracellular Na^+ resulting from inhibition of the membrane Na^+,K^+-ATPase activity leads to an increase of Na^+/Ca^{2+} exchange with a rise in intracellular Ca^{2+} and that neuronal depolarization sequentially activates an influx of Ca^{2+} into nerve cells (Landis & Putney 1979; Gill 1982). The elevation of cytosolic Ca^{2+} will inhibit Na^+,K^+-ATPase activity and by so doing trigger the transmitter release mechanism. Opiates have been suggested to depress neurotransmitter release by reducing the calcium entry into nerve cells (Cerreta et al 1977; Göthert et al 1979; Guerrero-Munoz et al 1979; Pillai & Ross 1986). Although several investigators have studied the effects of opioids on the membrane Na^+,K^+-ATPase activity in-vitro, consistent results have not yet been obtained (Desaiah & Ho 1977, 1979; Wan-Kan & Hosein 1981; Gandhi & Daginawala 1985; Hàjek et al 1985b; Pillai & Ross 1986). In the present study, we detected a statistically significant stimulatory action of morphine on synaptosomal Na^+,K^+-ATPase activity over a restricted range of free Ca^{2+} concentrations. The membrane Na^+,K^+-ATPase activity was depressed by Ca^{2+} in our experiment, as in those reported by many investigators. The stimulatory action of morphine on membrane Na^+,K^+-ATPase activity might result from its ability to block the Ca^{2+}-induced inhibition of the enzyme over the restricted range of free Ca^{2+} concentrations. At high Ca^{2+} levels, the effect of morphine might not be powerful enough to overcome the stronger inhibitory effects of Ca^{2+} (Fig. 2A). The inconsistency between our and the others' data might be due to the different experimental conditions, especially the difference in free Ca^{2+} concentrations in the medium.

Antagonism by naloxone of morphine stimulatory action on Na^+,K^+-ATPase activity indicates that this enzyme may serve as opiate receptors or the active site of the enzyme may be located near opiate receptor sites on the synaptic membrane. Additional work is clearly needed to explore the above-mentioned possibilities.

From our results, we postulate that the inhibitory effect of morphine on noradrenaline release from the rat cerebral cortex may partly be explained by the stimulatory action of morphine on the membrane Na^+,K^+-ATPase activity.

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Letter to the Editor

Classification of percutaneous penetration enhancers: a conceptional diagram

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The stratum corneum has long been considered a major barrier to penetration of topically applied chemicals (Marzulli 1962; Vinson et al 1965). Many compounds have low permeabilities through skin. Consequently, some transdermal drug delivery systems have utilized enhancers to accelerate drug permeability (Barry 1983). Percutaneous delivery enhancers may offer a means of increasing drug permeation; at present, a cohesive theoretical basis for choosing and formulating such agents is incomplete.

If quantitative structure activity relationships could be developed for percutaneous enhancers, it would facilitate selection of chemicals to be screened as putative enhancers. We propose a classification of chemicals using a conceptional diagram to estimate their potential as enhancers. This diagram was originally developed to predict the properties of organic compounds (Fujita 1954) and has been applied in diverse research (Kouda 1984); e.g. the level of bioaccumulation of organic compounds in fish can be predicted (Matsuo 1979, 1980a, b, 1981).

Fujita (1954) determined an organic and inorganic value for each compound of interest depending on its structural components. These values are based on boiling point. He assumed the organic properties depend on carbon atoms and inorganic character depends on substituted groups. An organic value is derived by summing up the number of the carbon atoms, one carbon atom having a value of 20. Other organic and inorganic values were calculated using Fujita's table (Fujita 1954; Kouda 1984). We calculated the organic and inorganic values for chemicals reported to enhance percutaneous penetration (Table 1). Fig. 1 depicts the location of these cutaneous enhancers, when the organic value is plotted against the inorganic value.

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The enhancers are located in two different areas on the diagram. Area I includes ethanol, propylene glycol, *N*-methyl pyrrolidone, and dimethyl sulfoxide and area II includes 1-dodecylazacycloheptan-2-one (Table 1, no. 14), oleic acid, and lauryl alcohol. The different locations suggest that the chemicals in the two groups may have different physicochemical properties.

Table 1. Organic and inorganic values of percutaneous penetration enhancers.

Enhancer	Organic	Inorganic
1. Water	0	100
2. Ethanol (a)	40	100
3. Propylene glycol (a)	60	200
4. *N,N*-Dimethyl acetamide (a)	80	200
5. *N,N*-Dimethyl foramide (a)	60	200
6. 2-pyrrolidone (a)	80	145
7. *N*-Methyl pyrrolidone (a)	100	145
8. 5-Methyl-2-pyrrolidone (a)	100	145
9. 1,5-Dimethyl-2-pyrrolidone (a)	120	145
10. 1-ethyl-2-pyrrolidone (a)	120	145
11. 2-Pyrrolidone-5-carboxylic acid (a)	100	295
12. Dimethyl sulfoxide (b)	80	140
13. Oleic acid (c)	360	152
14. 1-Dodecylazacycloheptan-2-one (d)	360	145
15. *N,N*-Dimethyl-m-toluamide (e)	240	215
16. n-Decyl methyl sulfoxide (f)	260	140
17. Lauryl alcohol (g)	240	100
18. Lauric acid (g)	240	150
19. Isopropyl myristate	330	60

Organic and inorganic values of enhancers were calculated from Fujita's table (Fujita 1954; Kouda 1984). Enhancers are from: a (Barry 1983), b (Chandrasekaran et al 1977), c (Cooper 1984), d (Stoughton 1982), e (Windheuser et al 1982), f (Cooper 1982), g (Aungst et al 1986).