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Effects of [D-Ala^2-*O*-benzyl-Ser^5]-enkephalin on CA 1 field potentials in rat hippocampal slices

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ABSTRACT [D-Ala^2-*O*-benzyl-Ser^5]-enkephalin (DABS) showed 2 effects upon the evoked potentials recorded in CA 1 cell body layer: 1) production of naloxone-reversible additional population spike (PS), and 2) enhancement of the primary PS which was partially reversed by naloxone in most experiments. The differential antagonism of the two effects by naloxone suggested that they might be mediated by different opiate receptor subtypes. The mechanism of augmentation by DABS of the evoked potentials could be attributed to disinhibition as judged from the paired-pulse paradigm.

KEY WORDS [D-Ala^2-*O*-benzyl-Ser^5]-enkephalin; naloxone; hippocampus; evoked potentials

Opiate receptors and enkephalin-like immunoreactivity have been found in the rat hippocampus. Although exogenously applied opiates and opioid peptides exert naloxone-reversible inhibition in an overwhelming majority of brain regions, hippocampus is one of a few exceptions where they exhibit apparent excitatory action. Moreover, the opioid-induced excitation in the hippocampus is most likely achieved by suppression of the inhibitory interneurons, i. e. disinhibition[1].

DABS has been shown to possess a much higher potency in inhibiting mouse vas deferens contraction evoked by electric field stimulation as compared to that in guinea pig ileum assay, suggesting that the enkephalin analog is a highly specific agonist for the δ-opiate receptor subtype[2]. However, the receptor binding assay carried out in our laboratory indicated that DABS showed an affinity for the δ-receptor subtype only about 10 times higher than that for the μ-receptor subtype[3]. Hippocampal slice preparations have been used extensively to study the opioid action. Moreover, both μ- and δ-receptors have been demonstrated in the hippocampus in a receptor binding study[4]. Therefore, it seems worthwhile to test the effects of DABS on the electrical activity of hippocampus. The present work, as the final part of a DABS research series, presents an analysis of the effects of the enkephalin analog on field potentials in CA 1 region of rat hippocampal slices maintained *in vitro* in comparison with the effects of some opiates.

MATERIALS AND METHODS

Hippocampal slices were prepared from rats (148±SD 36 g), anaesthetized with ether. After having removed, the brain was cooled in 4℃ saline for 1 min. The hippocampus was dissected out, 400 μm thick transverse slices were cut with a tissue chopper and transferred to a Haas' chamber where they were perfused with artificial cerebrospinal fluid (ACSF) at 32-34℃. The flowrate was 1 ml/min. Warmed, moistened 95% O_2 + 5% CO_2 was continually deflected over the surface of the slices[5]. The ACSF contained (mmol/L): NaCl 124, KCl 2, KH_2PO_4 1.25, $MgCl_2$ 2, $CaCl_2$ 2, $NaHCO_3$ 26, glucose 10 and was equilibrated with the gas mixture which maintained a pH value of 7.4. Slices were incubated

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for 1–2 h prior to use.

Conventional electrophysiological methods were used for stimulating and recording. Stimulus pulses were delivered at 0.5 Hz via bipolar tungsten electrodes or a monopolar stainless steel electrode, and were isolated with current constant. Field potentials were recorded from CA 1 cell body layer (stratum pyramidale) using glass microelectrodes (resistance 5–10 MΩ) filled with 4 mol/L potassium acetate. CA1 pyramidal cells were driven orthodromically by stimulation of Schaffer collaterals in stratum radiatum, the afferent fibres to the cells (Fig 1–A, B). Signals were amplified, displayed on a Nicolet 2090 oscilloscope, and photographed or saved on floppy disks for subsequent analysis.

The field potentials recorded in CA 1 pyramidal cell body layer following stimulation of Schaffer collaterals consisted of a slow positive potential upon which, at sufficient intensity of stimulus, a negative PS was superimposed (Fig 1–C). The slow potential has been interpreted as extracellularly recorded excitatory postsynaptic potential (EPSP) generated in the apical dendrites. The negative PS has been demonstrated to be a synchronous discharge of many neighbouring neurons and represents a measure of the over–all excitability of CA 1 pyramidal cell population[6].

In order to assess the effects of drugs over the entire functional range of the neuron population, the input/output relation was investigated. When responses stabilized in control ACSF, the stimulus current for pulses of 30 μs duration was usually adjusted to just above the threshold for generating PS, and kept constant for the subsequent experiment. The size of the stimulus (input, I) was altered by varying the pulse duration from 10 to 90 μs, and the responses to the stimuli (output, O) were sampled to construct I/O curves (n = 2, at each stimulus size).

The following parameters of the evoked field potential were measured for generating I/O curves. 1) PS amplitude was calculated as the average of the maximum initial positivity to the peak spike negativity and the peak negativity to the following maximum positivity. The amplitude

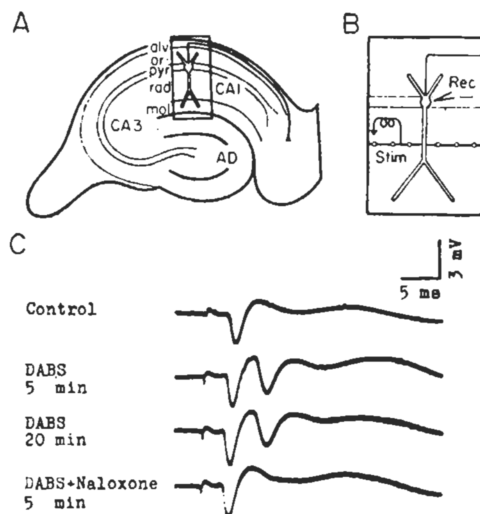

Fig 1. Activity in cell body layer. A) Transverse hippocampal slice with the main regions (CA 1, CA 3 and the dentate area, AD) and the layers of the CA 1 indicated. Abbreviations: alv = alveus, or = stratum oriens, pyr = stratum pyramidale, rad = stratum radiatum, mol = stratum moleculare. B) The boxed–in area in A) shown in greater detail. Stim = orthodromic stimulation of Schaffer collaterals, the afferent fibres in radiatum. Rec = recording of the field potential in the cell body layer. C) The responses to orthodromic stimulation recorded extracellularly at the cell body layer before and after perfusion with DABS 50 μmol/L and naloxone 50 μmol/L. Note the rapid onset of the effects for both drugs. The sample was taken from one of the early experiments in which supramaximal stimuli (2 times the threshold intensity) were used, therefore no detectable enhancement of the primary PS was seen after the addition of DABS.

of the additional PS which appeared after drug application was obtained in a similar manner. 2) PS latency was taken as the time from the stimulation artifact to peak spike negativity. 3) Extracellular EPSP amplitude was measured as a positive potential at an arbitrarily chosen latency, fixed for each experiment and not more than 2 ms after onset to avoid distortion by the subsequent PS.

DABS was synthesized in our institute. Morphine hydrochloride and codeine phosphate were purchased from Qinghai Pharmaceutical Factory, and naloxone hydrochloride from Red Flag Pharmaceutical Factory of Shanghai Medical University. All drugs were dissolved in ACSF just before use, and administrated by perfusion. Because slices in the chamber were exposed to the drugs at the same time, only the best slice was chosen to be used in each experiment.

RESULTS

All naive slices used in the present work showed a single PS of 3 mV or more in response to an orthodromic stimulus of less than 50 μA (50 μs, 0.5 Hz) and no additional PS over the entire range of the I/O curve.

In early experiments the field potentials evoked by stimuli of twice the threshold current were taken as control. DABS 1–50 μmol/L elicited additional PS with no significant change in the size of the primary PS in 9 out of 10 slices tested (Fig 1–C). Sometimes, the secondary PS was approximately equal to the primary PS with respect to the amplitude. The enhancement of the primary PS was only observed in one slice in which no additional PS appeared following application of DABS 3 μmol/L. The DABS effects had an onset within 5 min, and the peak was reached at 20–30 min. Perfusion with naloxone (in the same concentration as those for DABS) always abolished the DABS-induced additional PS within 5–10 min. Morphine and codeine shared the effects of DABS, however, the concentrations needed to induce comparable effects were 2–10 times higher than that for DABS.

The quantitative analysis of the DABS effects was accomplished by means of generating I/O curves in the other 12 slices. Fig 2 is a sample representative of such analysis. There was a considerable increase in the size of the primary PS coupled with the appearance of the secondary PS over the entire range of the stimulus intensities 20 min following bath application of DABS 1 μmol/L (panels A, D). In contrast to the significant change in PS, the extracellular EPSP was only slightly enhanced at the higher intensities of stimulus (panel B). Therefore, when the primary PS amplitude is plotted as a function of the corresponding amplitude of the extracellular EPSP, DABS caused a significant leftward shift of the curve (panel C), indicating that a considerably large fraction of the pyramidal cells was brought to discharge at a given size of the extracellular EPSP, i. e. so-called 'E-S potentiation'[7]. Naloxone 1 μmol/L abolished DABS-induced secondary PS within 10 min. However, the drug only partially reversed the increase in the amplitude of the primary PS at the lower intensities of stimulus, did not affect or even enhanced it slightly at the higher intensities 20 min after perfusion. DABS had no detectable effect on the latency of the primary PS, and surprisingly, naloxone markedly reduced it (panel E). Similar results were obtained from 7 other slices, in which the concentrations of DABS tested ranged from 0.1–50 μmol/L. There were some discrepancies between these experiments. Thus, no additional PS appeared over the entire range of the I/O curve with lower concentrations of DABS in 4 of the 8 slices. In one of them, com-

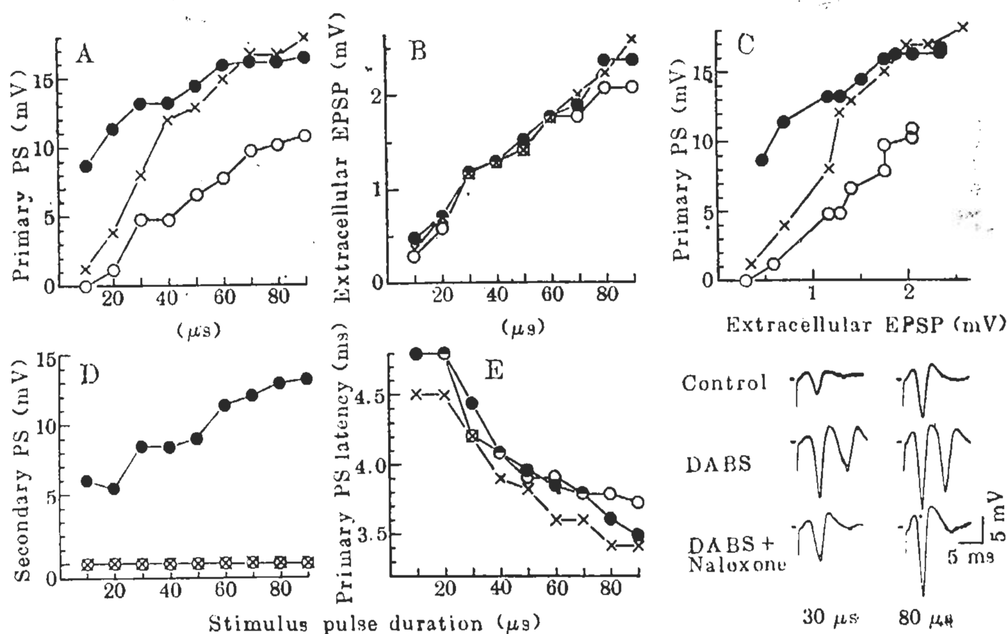

Fig 2. I/O analysis from one slice. ○ Control, ● DABS (1 μmol/L) 20 min. × DABS (1 μmol/L) + naloxone (1 μmol/L) 20 min. n = 2 sweeps. The records for 2 stimulus intensities (30, 80 μs) were present at the bottom right. Each record Consists of 2 superimposed sweeps.

plete reversal of DABS-induced enhancement of the primary PS was obtained with a naloxone concentration two times higher than that for DABS. In addition, DABS also enhanced the PS evoked by antidromic stimulation of alveus, the efferent fibres from CA 1 pyramidal cells (n = 1). Moreover, I/O curves were constructed for the field potentials in CA 3 region evoked by stimulation of the mossy fibres, as well as in the dentate area by stimulation of the perforant path (n = 3). DABS induced similar effects as in CA 1 region.

A homosynaptic paired-pulse (conditioning-test) paradigm has been employed by several authors to explore the mechanisms involved in the excitatory action of opioids on the hippocampus[8-10]. The ratio of the test response versus the conditioning response (T/C) has been considered to be an indication of sequential inhibition or sequential facilitation characteristic of the hippocampal circuitry, depending upon the inter-pulse interval. In the present study double-pulse stimulation with identical intensity was delivered to Schaffer collaterals, and the effects of DABS on both the conditioning PS and the test PS were examined, and then the ratio calculated. As described above, DABS elicited additional PS in most slices tested, and this may distort the test PS particularly at short inter-pulse intervals in the paired-pulse experiment. Therefore, only the slices which did not show DABS-induced additional PS in the preceding I/O analysis were chosen for the paired-pulse paradigm. As shown in Fig 3, perfusion of the slice with DABS (2 μmol/L) caused: 1) an enhancement of the conditioning PS (from 7.1 to 8.4 mV), 2) a suppression of sequential inhibition (A, B, the ratio increased from 0.46 to 0.75), and 3) an increase of sequential facilitation (C, D, the ratio from 1.16 to 1.40). Similar effects were observed in 3 out of 4 slices tested.

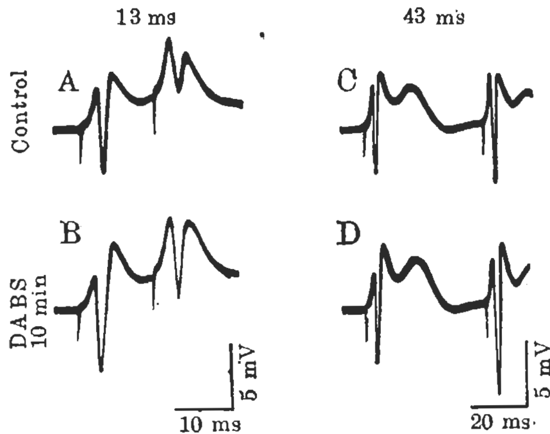

Fig 3. Paired-pulse inhibition (A) and facilitation (C) were obtained by delivery of paired-pulse stimulation to stratum radiatum at inter-pulse intervals of 13 and 43 ms, respectively. The inhibition was reduced (B), and the facilitation enhanced (D) 10 min following application of DABS 2 μmol/L. Naloxone 3 μmol/L reversed the effects of DABS (not shown).

DISCUSSION

In the present study DABS showed two effects upon the evoked field potentials recorded in CA 1 cell body layer of rat hippocampus: 1) production of naloxone-reversible additional PS, and 2) enhancement of the primary PS which was partially reversed by naloxone in most experiments. Both effects reflect an increase in the over-all excitability of CA 1 pyramidal cell population. Our results are consistent with those reported previously by authors who tested opiates and other opioid peptides on the same preparations[1,8,9].

Two modes of action by which opioids might produce the excitatory action have been proposed, namely disinhibition[11,12], and increased release of excitatory transmitters[13]. Our results are in favour of the mode of disinhibition. Firstly, a marked enhancement of PS contrasts strikingly with little change in the size of extracellular EPSP following DABS application. As a result, 'E-S potentiation' is exhibited to varying degree in all slices tested, indicating that the action is due to a great increase in the excitability of the postsynaptic CA 1 pyramidal cells, but not to presynaptic facilitation. Secondly, the fact that DABS enhances the antidromically-activated PS supports the same notion. Finally, more direct evidence is obtained from the paired-pulse paradigm. DABS affects the paired-pulse function mainly at the shorter inter-pulse intervals at which sequential inhibition (due to recurrent feed-back, or feed-forward inhibition by GABAergic interneurons) plays a predominant role[8-10]. The inhibition is obviously attenuated by DABS as shown in Fig 3.

Based upon I/O analysis we have noticed differential naloxone antagonism between the two effects of DABS: the additional PS is readily reversed by, and the enhancement of the primary PS is relatively resistent to naloxone in the concentrations approximately equal to those for DABS. The reason for such a phenomenon remains uncertain. However, the notion that there may be two separate mechanisms, one for the production of additional PS, the other for the increase in the size of the primary PS has been proposed, and it is supported by the fact that significant tolerance developed to the former but not to the latter[14]. It is well known that naloxone is a more potent antagonist at the μ-receptor subtype than at the δ-subtype. If the production of additional PS and the enhancement of the primary PS were assumed to be mediated by μ- and δ-receptor subtypes, respectively, the difference in naloxone antagonism could be readily explained.

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[D-丙^2-*O*-苯甲基-丝^5]脑啡肽对大鼠海马薄片CA1场电位的影响

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提要　[D-丙^2-*O*-苯甲基-丝^5]脑啡肽(DABS)对CA 1锥体层记录的场电位有两种作用：1)诱发附加的群峰，2)增大原有的群峰。前者易被纳洛酮逆转，后者在多数实验中仅部分地被纳洛酮逆转，提示这两种作用可能由不同的阿片受体亚型介导。输入/输出(I/O)关系分析指出DABS对胞外兴奋性突触后电位(EPSP)几乎无影响，给药后胞外EPSP-群峰幅度曲线左移，使同样大小的胞外EPSP产生较大的群峰，说明DABS的作用是提高突触后锥体细胞的兴奋性，不是增加突触前兴奋性递质的释放。双脉冲实验进一步证明DABS增大CA 1场电位的机理是脱抑制作用。

关键词　[D-丙^2-*O*-苯甲基-丝^5]脑啡肽；　纳洛酮；海马；　诱发电位