EFFECT OF THE β-CARBOLINE DERIVATIVE FG7142 ON INHIBITION IN HIPPOCAMPAL SLICES

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Derivatives of β-carboline, which include methyl-β-carboline-3-carboxamide (FG7142), are high-affinity ligands of benzodiazepine receptors (BDR) [3]. However, the pharmacologic activity of these compounds is opposite to that of the benzodiazepines (BD). It has been shown, for instance, that FG7142, on peroral administration to man, causes attacks of acute anxiety, which can be terminated by BD [4]. In addition, in certain biochemical tests the β-carbolines exhibit properties opposite to those of BD [3]. The writers previously described the effect of some β-carboline derivatives on evoked activity of hippocampal neurons [2]. This paper describes a more detailed analysis of the action of FG7142 with extracellular and intracellular recording.

EXPERIMENTAL METHOD

Experiments were carried out on surviving hippocampal slices of Wistar rats weighing 70–100 g, by the method described previously [1]. Recording electrodes were filled with 0.9% NaCl for extracellular recording or with 2M potassium citrate for intracellular recording (the resistance of the electrodes was 1-3 and 40-60 MΩ respectively). Activity was recorded extra- and intracellularly in area CA1.

To measure the degree of inhibition quantitatively, paired stimulation of Schaffer's collaterals (SC) with equal square pulses of direct current (5-20 V, 0.2 msec, 0.1 Hz) was used. The amplitudes of two population spikes (PS) in response to these stimuli were recorded extracellularly. The measure of inhibition was the ratio A2/A1 × 100%, where A1 denotes the amplitude of PS in response to the first stimulus, A2 the amplitude of PS in response to the second stimulus. The interval between pulses varied in each experiment from 20 to 100 msec.

All substances for application were added to the flow of liquid up to a total concentration of 5 μM. Working concentrations were prepared by diluting concentrated solutions of the substances (2×10^{-2} M) in ethanol with the perfusing solution. In the control, the corresponding amounts of ethanol was added to the flow of liquid. Diazepam and RO15-1788 (from Hoffman-LaRoche, Switzerland) and FG7142, synthesized in the Laboratory of Neurochemistry, Brain Institute (Head, Candidate of Chemical Sciences V. P. Demushkin) [2], were used. The structural formula of the compounds is given in Fig. 1.

EXPERIMENTAL RESULTS

The writers showed previously that the action of FG7142 on PS in hippocampal area CA1 was to increase the amplitude of PS by 10–20%; in nearly every case extra PS appeared. As a rule, after the beginning of rinsing the effect of FG7142 strengthened and continued for a long time during rinsing [2]. To test the hypothesis that the action of FG7142 is mediated by BDR, FG7142 (5 μM) was applied together with diazepam (5 μM) or with RO15-1788 (5 μM), a specific antagonist of BD. As will be clear from Fig. 1, I, during combined application of FG7142 and diazepam no appreciable changes took place in PS. During rinsing with physiological saline, in some cases there was a small (by 5-10%) decrease in PS (in three of seven experiments), but in the rest PS was unchanged. After 30 min of rinsing to remove both

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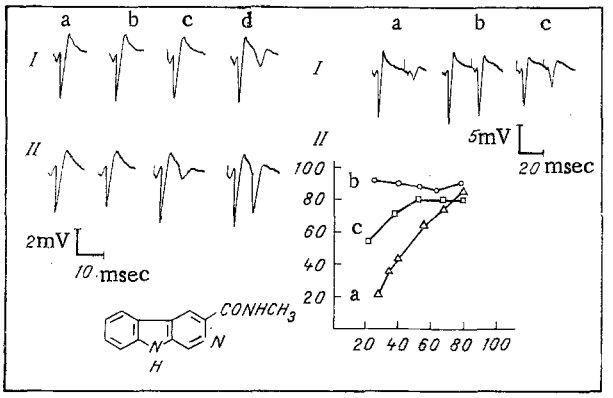

Fig. 1. Fig. 2.

Fig. 1. Blockade of facilitating effect of FG7142 (5 μM) on evoked potential (EP) (individual case) by diazepam and RO15-1788 (5 μM): a) control EP; b) EP after 15 min of combined application of FG7142 and diazepam (I) or FG7142 and RO15-1788 (II); c) EP after rinsing out substances for 30 min; d) EP after application of FG 7142 for 15 min. Structural formula of FG7142 shown below.

Fig. 2. Action of FG7142 (5 μM) and diazepam (5 μM) on EP during paired stimulation (typical individual case). I) Action of FG7142 and diazepam on EP with constant interval between pulses: a) control, b) EP after application of FG7142 for 15 min; c) EP after rinsing out FG7142 for 30 min followed by application of diazepam for 15 min; II) effect of FG7142 and diazepam on EP with different interval between stimulating pulses. Abscissa, interval between stimuli (msec); ordinate, ratio A2/A1 × 100%. a) Control; b) application of FG7142 for 15 min; c) after rinsing out FG7142 for 30 min followed by application of diazepam for 15 min.

substances, FG7142 (5 μM) was applied separately. Application of FG7142 caused the appearance of an additional PS in all seven experiments and an increase in amplitude of the first by 10-15% (Fig. 1). Experiments with combined application of FG7142 (5 μM) and RO15-1788 (5 μM) were conducted by a similar scheme. In this case FG7142 had no effect. In two of five experiments PS was reduced (13 and 24% respectively). Unlike in the previous series of experiments with diazepam, during rinsing signs of action of FG7142 were observed in three of five experiments (an increases in PS, the appearance of an additional PS). Subsequent separate application of FG7142 (5 μM) enhanced these effects (Fig. 1).

The absence of an excitatory effect of FG142 on combined application with diazepam and RO15-1788 suggests that the action of the compound in this case is mediated through BDR. It is an interesting fact that RO15-1788 in half of the experiments did not affect the development of after-facilitation of the evoked potential (EP) under the influence of FG7142. On combined application of FG7142 and diazepam, followed by rinsing out both of them, facilitation was not observed. This difference in the effectiveness of inhibition of the after-effects by diazepam and RO15-1788 can probably be explained on the ground that diazepam, unlike RO15-1788, by interacting with BDR, makes GABA-inhibition more effective. The higher level of inhibition also was maintained after the diazepam had been rinsed out, which prevented the development of after-effects of FG7142 when applied together with diazepam.

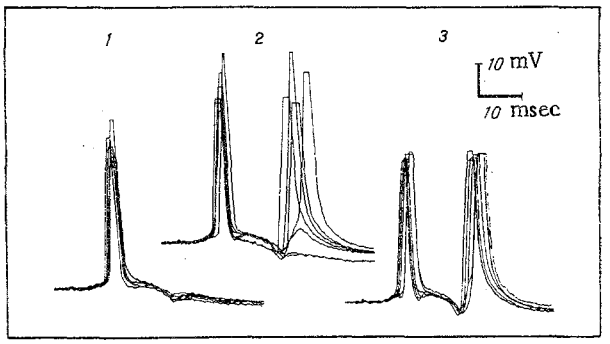

Fig. 3. Action of FG7142 (5 μM) on evoked unit activity during intracellular recording (individual case): 1) control; 2) application of FG-7142 for 5 min; 3) rinsing for 15 min.

To assess the action of FG7142 on inhibitory processes in the hippocampus paired stimulation of SC was used (seven experiments). The reduction in amplitude of the second PS compared with the first in the control can be explained by activation of inhibitory interneurons by the first stimulus, so that the second PS was more exposed than the first GABA-inhibition. The effect of FG7142 (5 μM) on the extracellular EP during paired stimulation is shown in Fig. 2, I: FG7142 incerased the amplitude of the second PS considerably (four-fivefold); the amplitude of the first PS in this case was increased by 10-20%. This effect can be interpreted as abolition of the inhibitory effect of GABA. The effect of FG7142 on inhibition with different intervals between stimuli is shown in Fig. 2, II. FG7142 (5 μM) abolished inhibition induced by the first stimulus, whatever the interval. Application of diazepam immediately after rinsing out FG7142 in three of seven experiments partially neutralized the effect of FG7142, and restored inhibition (Fig. 2). The effect of FG7142 on EP during paired stimulation thus consisted of a marked increase in amplitude of the second PS, with only a weak effect on the first. The action of this compound on inhibition may be mediated by several mechanisms: 1) a reduction in activity of inhibitory interneurons; 2) a decrease in the number of GABA-receptors on the postsynaptic membrane; 3) a decrease in the effectiveness of GABA as a result of conformational changes in the GABA receptor — BDR — chloride channel complex. Diazepam partially neutralized changes induced by FG7142, probably by affecting these mechanisms in the opposite way.

Intracellular recording was used to study the action of FG7142 on parameters of a single neuron. Using a stimulus of below-threshold strength for action potential (AP) induction, the effect on membrane potential (MP) and on the inhibitory and excitatory postsynaptic potential was not recorded in any of the five traces. With above-threshold stimulation, application of FG7142 (5 μM) led initially to the appearance of local depolarization immediately after AP, in place of which, if application of substance continued, a second AP appeared (Fig. 3). During simultaneous extra- and intracellular recording the latent periods of the second (extra) PS and the second AP coincided. During rinsing the intracellular effect of FG7142 was strengthened, as shown by the fact that two AP were generated in response to each stimulus during rinsing, whereas during application some of the intracellular responses consisted of one AP and subthreshold depolarization (Fig. 3). The nature of this depolarization is not quite clear. It may reflect recurrent excitation which, under ordinary conditions, is suppressed by recurrent inhibition, i.e., it may be the result of disinhibition, due to the action of β-carboline. Another explanation could be that FG7142 acts directly on the membrane of pyramidal neurons, facilitating conduction of dendritic spikes into the soma.

Changes in the elctrophysiological parameters of the hippocampal slices under the action of the high-affinity BDR ligand FG7142, which induces an anxiety state in man and animals when administered *in vivo* [4, 6], were studied in the present investigation. The limbic system probably participates in anxiety formation [5]. The effectiveness of the action of an anxiogen, such as FG7142, on hippocampal unit activity is further confirmation of the participation of this structure in the development of anxiety. The possibility cannot be ruled out that the hippocampus is one of the main points of application of the activity of FG7142, but to test this hypothesis the action of this substance on other brain structures must be studied. Reduction of the effectiveness of inhibitory hippocampal synaptic connections is perhaps one of the essential mechanisms involved in the genesis of an anxiety state. Inhibition of the

inhibitory system under the action of the action of the anxiogen FG7142 is expressed as changes in evokedneuronal activity in hippocampal slices. Changes of this kind, characterized by the apprearance of additional PS and AP, can be regarded conjecturally as electrophysiological correlates of anxiety.

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