TWO TYPES OF GABA RECEPTORS IN THE INTACT OLFACTORY BULB AND PRIMORDIAL HIPPOCAMPUS OF THE FROG: PHARMACOLOGIC DATA

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KEY WORDS: olfactory bulb; primordial hippocampus; GABA receptors

The well known role of GABA in the vertebrate CNS and the fact that many neurotropic drugs used in clinical practice are GABA derivatives have given rise to great interest in the study of the physiological role and localization of molecular GABA receptors in various brain structures. In recent years a new type of GABA receptors ($GABA_B$-receptors), insensitive to many GABA-mimetics, and also to bicuculline [9], has been found. Great importance is attached to $GABA_B$-receptors in the mechanism of presynaptic inhibition. Their specific agonist is (-)-baclofen [β-(p-chlorophenyl)-γ-aminobutyric acid], which is inactive against classical $GABA_A$-receptors [9, 12]. Most investigations of $GABA_A$- and $GABA_B$-receptors have been diverted to the study of their functional role in different parts of the brain and have been conducted on *in vitro* models. Nevertheless, the final solution to these problems necessitates experiments *in vivo* [10].

This paper describes an attempt to demonstrate pharmacologically two types of GABA-receptors in the intact nervous system of *Rana temporaria*. The effect of baclofen, an agonist of GABA-receptors, of muscimol, an agonist of $GABA_A$-receptors [8], and of bicuculline, an antagonist of $GABA_A$-receptors [11] on evoked potentials in the olfactory bulb and primordial hippocampus was investigated during electrical stimulation of the afferent olfactory input. The results are evidence of the presence of both types of GABA-receptors in the above-mentioned brain structures and of differences in their localization in the olfactory bulb.

EXPERIMENTAL METHOD

Experiments were carried out at room temperature (20-23°C) on mature male frogs (*Rana temporaria*) in the fall and winter. The animals were immobilized with diplacin* (10-15 mg/kg) and, after local anesthesia (procaine) the skull was opened, to expose the surface of the hemispheres of the forebrain (the primordial hippocampus), the olfactory bulbs, and the proximal segments of the olfactory nerves. EP were recorded from the surface of the olfactory bulb and primordial hippocampus by a monopolar technique with a silver electrode (diameter 0.1 mm): the reference electrode (a silver disk) was placed inside the mouth. The orthodromic and antidromic EP (OEP and AEP respectively) of the olfactory bulb were obtained by electrical stimulation of the lipsilateral olfactory nerve and the olfactory tract (square pulses: 15-40 V, 0.1-0.2 msec) through bipolar nichrome electrodes (interelectrode distance 0.2-0.3 mm). The CEP of the primordial hippocampus was obtained in a similar manner during stimulation of the rostral part of the ipsilateral olfactory bulb. Biopotentials were recorded by means of a standard set of electrophysiological apparatus.

(±)-Baclofen (from Ciba-Geigy, Switzerland) and (+)-bicuculline (from Sigma, USA) were dissolved beforehand in 0.1 M HCl to a concentration of 10-15 mM. For dilution to the final concentration Ringer's solution for cold-blooded animals, made up in 10 mM Tris-HCl, pH 7.3, was used. Muscimol (from Sigma) was dissolved directly in Ringer's solution. Ringer's solution with the addition of the corresponding amounts of HCl or sucrose was used in control experiments. The solutions were applied (0.5-1.0 µl) by calibrated micropipets in the region of EP derivation.

*1,3-di(β-platyneciniumethoxy)benzene hydrochloride.

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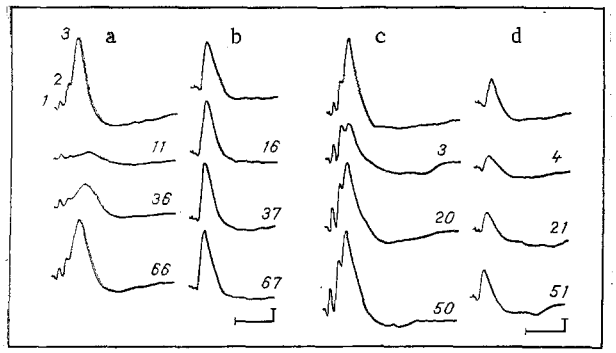

Fig. 1

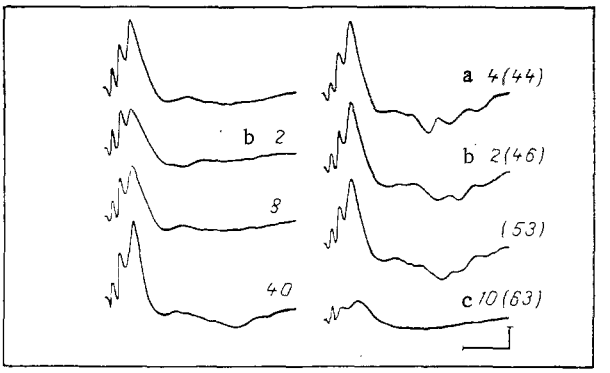

Fig. 2

Fig. 1. Action of baclofen (a, b) and muscimol (c, d), on OEP (a, c) and AEP (b, d) of olfactory bulb. Concentrations of baclofen and muscimol 10^{-5} traces (a-d) represent control. 1, 2, 3) Negative components of OEP (in upward direction). Numbers indicate time after application (in min). Calibration: 250 μV, 100 msec.

Fig. 2. Changes in OEP of olfactory bulb in response to application of muscimol (10^{-5} M), baclofen (10^{-5} M), and bicuculline (10^{-3} M). Successive recording during one experiment. Time (in min) after first application of muscimol shown in parentheses. Numbers indicate time (in min) after subsequent application of bicuculline (a), muscimol (b), and baclofen (c). Calibration: 250 μV, 100 msec.

EXPERIMENTAL RESULTS

Just as in other vertebrates, three principal negative components were identified in the OEP of the olfactory bulb of the frog recorded from the rostral pole: a fast presynaptic peak (composite spike to olfactory nerve fibers), and the 1st and 2nd postsynaptic components (Fig. 1a), reflecting monosynaptic and polysynaptic excitation of the neurons respectively [2]. The principal negative wave of the AEP (Fig. 1b) was similar in nature to the second postsynaptic component of the OEP [3].

Baclofen (10^{-6}-10^{-4} M) and muscimol (10^{-5}-10^{-4} M) caused well-marked but unequal changes in EP of the olfactory bulb, which were reversible and dose-dependent in character. Baclofen, in a concentration of 10^{-5} M, caused considerable (by 60-90%) and prolonged (40-90 min) depression of both postsynaptic components of OEP, but had virtually no effect on the presynaptic component (Fig. 1a). The amplitude of the AEP did not change significantly; mainly it increased (Fig. 1b). The larger dose of baclofen (10^{-4} M) caused complete suppression, lasting several hours, of the postsynaptic components of the OEP. In a concentration of 10^{-6} M baclofen caused a small decrease (by 20-40%) of these components of the OEP, only in individual experiments, followed by their rapid (10-20 min) recovery. After preliminary (6-10 min beforehand) application of bicuculline in a concentration of 10^{-4}-10^{-3} M the action of baclofen, in the doses tested, on EP of the olfactory bulb was virtually unchanged (Fig. 2).

Muscimol (10^{-4} M), unlike baclofen, caused parallel depression of the amplitude of the AEP and of the second postsynaptic component of the OEP in the olfactory bulb by 40-70% (Fig. 1c, d). An increase in amplitude of the presynaptic component of the OEP also was frequently observed, and this may have been connected with an increase in excitability of the axons of the olfactory nerve due to their depolarization (Fig. 1c). The action of muscimol in a lower concentration (10^{-5} M) was blocked by bicuculline only when used in the highest concentration (Fig. 2). Incidentally, inhibition of the spontaneous epileptiform discharge in the olfactory bulb also was observed when bicuculline was used in the highest concentrations (10^{-3} M).

The OEP of the primordial hippocampus (Fig. 3) was more complex in its component composition than OEP of the olfactory bulb. Bacolfen (10^{-5}-10^{-4} M) and muscimol (10^{-5}-10^{-4} M) caused reversible dose-dependent inhibition of the OEP of the primordial hippocampus (Fig. 3a, b). The first positive component (pointing downward in Fig. 3), however, could be very slightly increased in this case (by 5-15%). The depth and duration of inhibition of the OEP by baclofen were greater than when muscimol was mused in the same concentration. A larger dose of baclofen (10^{-4} M) caused virtually complete, rapidly developing (10-20 sec), and prolonged (for several hours) depression of all components of the OEP except the first positive component.

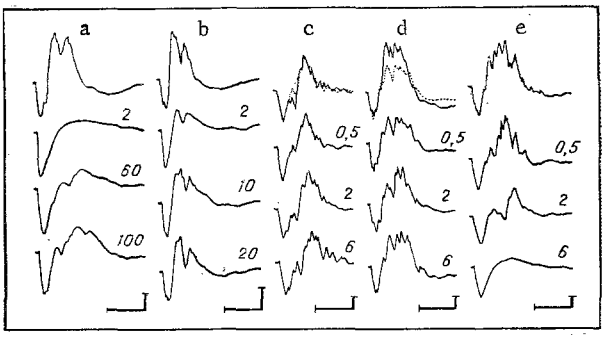

Fig. 3. Changes in EP of primordial hippocampus in response to applications of baclofen (10^{-4} M) and muscimol (10^{-4} M) separately and combined with bicuculline (5×10^{-4} M). Top traces: a, b) control; c, d, e) 6, 20, and 10 min respectively after application of bicuculline. Numbers indicate time (in min) after application of baclofen (a, e) and muscimol (b, d). Broken lines (c, d) represents OEP recorded 30 sec after application of muscimol. Calibration: 250 μV, 200 msec.

Bicuculline (10^{-6}-5×10^{-4} M) caused the appearance of additional high-frequency waves (Fig. 3, c-e) and of spontaneous epileptiform discharges in the OEP of the primordial hippocampus. This action of bicuculline appeared most stable in maximal doses 5-10 min after application and it continued for 60-90 min.

The action of muscimol, irrespective of its concentration, was virtually completely prevented by preliminary (5-10 min beforehand) application of bicuculline (5×10^{-4} M). With an increase in the interval between applications to 20-30 min the blocking action of bicuculline on the effect of muscimol was weakened (Fig. 3c, d), even though the modification of OEP and spontaneous epileptiform activity, induced by bicuculline, were most marked at this time. The effect of baclofen was completely preserved after application of bicuculline, but the rate of inhibition of the OEP was reduced (Fig. 3a, e).

These results are evidence of the existence of $GABA_A$-receptors both in the olfactory bulb and in the primordial hippocampus of *R. temporaria*. The effect of muscimol, which was inhibited in both structures by bicuculline, permits a conclusion to be drawn that is in good agreement with the known data on the pharmacology of synaptic inhibition in the olfactory bulb of lower vertebrates [15]. For the primordial hippocampus no analogous data are available, but biochemical analysis of the whole forebrain of the Anura has shown that it contains $GABA_A$-receptors [14]. Preliminary data [6] also indicates that a GABA-ergic system is present in the primordial hippocampus (the dorsomedial part of the forebrain). In the olfactory bulb extrasynaptic $GABA_A$-receptors are probably present also on axon terminals of the olfactory nerves, and this explains the axon depolarization observed under the action of muscimol and also of GABA [13]. However, their activation evidently does not play a role in inhibition of the input of the olfactory bulb during adequate stimulation [1, 4], because muscimol did not affect the magnitude of themonosynaptic component of the OEP (Fig. 1c). The difference in the intensity of the epileptogenic action of bicuculline in the olfactory bulb and the primordial hippocampus of the frog may be linked, not only with the characteristics of organization of excitatory-inhibitory connections, but also with differences in concentration of $GABA_A$-receptors in these structures.

Inhibition by baclofen of the monosynaptic component of the OEP in quite low concentrations indicates the presence of $GABA_B$-receptors on the axon terminals of the olfactory nerve in the olfactory bulb. The absence of parallel inhibition of the AEP means that cells of the bulb itself have no such GABA receptors or very few of them. Consequently, it is activation of $GABA_B$-receptors during accumulation of GABA in the glomeruli of the olfactory bulb that may lead to presynaptic inhibition of its input without the participation of synaptic transmission [4].

Our data are evidence of the existence of GABA-receptors in the primordial hippocampus. The high resistance of the first positive component of the OEP to baclofen, by analogy with the presynaptic component of the OEP of the olfactory bulb, is perhaps connected with its

presynaptic nature and, correspondingly, with the presynaptic localization of $GABA_B$-receptors in the primordial hippocampus. This problem requires special investigation, for $GABA_B$-receptors in the mammalian hippocampus may evidently be postsynaptic in their location also [7]. Slowing of the action of baclofen in the primordial hippocampus after application of bicuculline in a high concentration may be associated with the relative sensitivity of the $GABA_B$-receptors to bicuculline [5].

The results of the present investigation confirm data of recent neuropharmacologic investigations showing the presence of two types of GABA receptors, at different levels of the vertebrate brain, with differences in their localization and the mechanism of their inhibitory action. The writers have shown for the first time that $GABA_B$-receptors are present in the olfactory bulb, so that the concrete mechanisms of presynaptic inhibition in this structure can be identified. The presence of $GABA_B$-receptors in the primordial hippocampus enables the question of the presence of presynaptic inhibition in this forebrain structure of the Anura to be subjected to examination.

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SPECIFICITY OF ACTION OF PYRACETAM, PYRITINOL, AND CLEREGIL ON THE TRANSCALLOSAL EVOKED POTENTIAL

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Drugs with nootropic action affect the mental functions and many integrative processes of the brain, but do not exhibit psychotropic activity as revealed by a number of commonly used pharmacologic tests [3, 9-11]. Considering the data on the important role of the corpus callosum in learning and memory [4, 5], it can be postulated that the transcallosal evoked potential (TEP) is one of the most informative parameters for analysis of the specificity of the effect of drugs with a nootropic action, and which improve memory.

The aim of this investigation was to undertake a comparative study of the effect of drugs with a nootropic type of action on the TEP of the rabbit brain.

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