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Effect of $GABA_B$-receptor agonist and antagonist on whole-cell patch-clamp recorded rat PO/AH neurons

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ABSTRACT

Intracellular recordings were made from neurons in slices of the preoptic area/anterior hypothalamus (PO/AH) of rats, using whole-cell patch-clamp recording techniques to investigate the effects of the $GABA_B$-receptor agonist baclofen and $GABA_B$-receptor antagonist CGP 35348 on neuronal response characteristics. Baclofen decreased firing rate of the neurons, regardless of their type of temperature sensitivity. The decrease in firing rate during baclofen application was accompanied with significant membrane hyperpolarization and decrease of input resistances. The firing rate (in all type of PO/AH neurons) was increased by $GABA_B$-receptor antagonist CGP 35348 and this was accompanied with membrane depolarization and increase of input resistances. These findings were abolished when baclofen and CGP 35348 were applied simultaneously. Our results are step of understanding the complicated mechanisms of action of GABAergic agents on the level of central temperature controller – the neurons of the PO/AH. ! 2015 Trade Science Inc. - INDIA

KEYWORDS

$GABA_B$-receptor agonist;
$GABA_B$-receptor antagonist;
Whole-cell patch-clamp recording;
PO/AH neurons;
Brain slices.

INTRODUCTION

It is now more than 40 years since the establishment of gamma-aminobutyric acid (GABA) as an inhibitory neurotransmitter within the mammalian brain[1]. GABA receptors are functional very early in the hypothalamic development and are expressed widely in the hypothalamus[2]. However, GABA exerts its effects by acting at three distinct receptors, the $GABA_A$-, $GABA_B$- and $GABA_C$-receptor. $GABA_A$ and $GABA_C$ receptors form membrane channels (ionotropic receptors), while $GABA_B$ receptors belong to the family of G-protein-coupled receptors (metabotropic receptors)[3, 4]. The observations of Bowery et al.[5] introduced the $GABA_B$ receptors, which are not confined to the periphery but are clearly functional within the central nervous system. Whereas $GABA_A$-receptor is an ion channel (ligand-gated chloride ion channel superfamily), the $GABA_B$-receptor is coupled with G-protein and negatively linked to adenylyl cyclase. This might be related to different pathways of action by the $GABA_A$- and $GABA_B$-receptor agonists.

Many experimental studies suggest the participation of GABA in the processes of thermoregulation. Thermoregulation is the complex physiologic process involving both central and peripheral autonomic mechanisms. The primary thermoregulatory

center resides in the preoptic area of the anterior hypothalamus (PO/AH) and controls the balance between heat gain and heat loss. There are warm- and cold-sensitive neurons, as well as temperature-insensitive neurons in the PO/AH and they are known to be affected by local and peripheral temperatures. The neurons in PO/AH are supposed to build a neuronal network which takes part in the central control of body temperature[6].

Immunohistochemical research have reported GABAergic terminals and receptors on the neurons of PO/AH[7, 8].

In order to contribute to understanding the mechanism of action of GABAergic agents at cellular level, the effects of $GABA_B$-receptor agonist baclofen and $GABA_B$-receptor antagonist CGP 35348 on neuronal response characteristics of rat PO/AH neurons were studied, using brain slice preparation and whole-cell patch-clamp recording techniques.

MATERIALS AND METHODS

Whole-cell patch-clamp recordings

Slices (350 μm) from the PO/AH of male Sprague-Dawley rats (200-250g) were prepared and stored as previously described[9]. The tissue slices were constantly perfused with artificial cerebrospinal fluid (ACSF) consisting of (mM): 124 NaCl, 26 $NaHCO_3$, 10 glucose, 5 KCl, 2.4 $CaCl_2$, 1.3 $MgSO_4$ and 1.24 KH_2PO_4. The ACSF was oxygenated (95% O_2, 5% CO_2), heated to 37°C using a thermoelectric assembly and allowed to flow hydrostatically into the recording chamber at 2 ml/min. Tissue temperature was monitored by a thermocouple in the perfusion medium directly below the tissue slices.

Using whole-cell patch-clamp recording techniques[10], intracellular recordings were made with 2 μm tip glass pipettes. These microelectrodes had 3-5 MΩ resistances and were filled with a solution containing (mM): 130 potassium gluconate, 10 EGTA, 10 Hepes, 2 ATP, 1 $CaCl_2$ and 1 $MgCl_2$; pH 7.2-7.3, 295-300 mosmol $(kg)^{-1}$. The ground electrode was maintained at a constant temperature in an outer bath that was connected to the recording bath by a filter-paper bridge. As previously described[9] the liquid junction potential was experimentally determined to be 12.0 mV, and this value was subtracted from all recorded potentials. The recordings were made using an Axon Instruments 200A amplifier in the current clamp modes. Stimulation protocols were conducted using pCLAMP 6.0 software from Axon Instruments.

During each experiment, neuron integrated firing rate, membrane potential and tissue temperature were monitored and recorded on a polygraph and computer, using software Axoscope 9. Neurons were also noted for location within the PO/AH region, mainly in the medial preoptic area. Each neuron was recorded for 5 min to determine its firing rate at 37°C. The neuron was then tested for temperature sensitivity with a temperature cycle (duration 7-10 min) which covered the range 32-40°C. This range of temperature has been demonstrated to elicit appropriate and graded thermoregulatory responses in vivo for several mammalian models[6].

Neuronal thermosensitivity (impulses s^{-1} $^oC^{-1}$) was defined by the thermal coefficient (TC), i.e. the linear regression coefficient of firing rate plotted as a function of temperature, using criteria established in studies in the PO/AH[11]. A neuron with a thermal coefficient of 0.8 impulses s^{-1} $^oC^{-1}$ or greater was classified as a warm-sensitive neuron. Neurons were considered cold-sensitive if their thermal coefficiens were equal to or less than –0.6 impulses s^{-1} $^oC^{-1}$. All other neurons were classified as temperature-insensitive neurons.

Values are expressed as means±S.E.M. Multivariate analysis of variance with repeated measures was used to compare neuronal responses. MiniAnalysis Program for spike analysis and Clampfit 8 for resistance test analysis were used.

Substances and design of experiments

The $GABA_B$-receptor agonist R(+)-baclofen hydrochloride (1 μM) (Sigma-Aldrich GmbH) and the $GABA_B$-receptor antagonist CGP 35348 (100 μM) (Sigma-Aldrich GmbH), were diluted in ACSF just prior to the application. Before application of the test substances, the temperature sensitivity of a given neuron was determined using sinusoidal temperature stimulus. Superfusion of test substances baclofen

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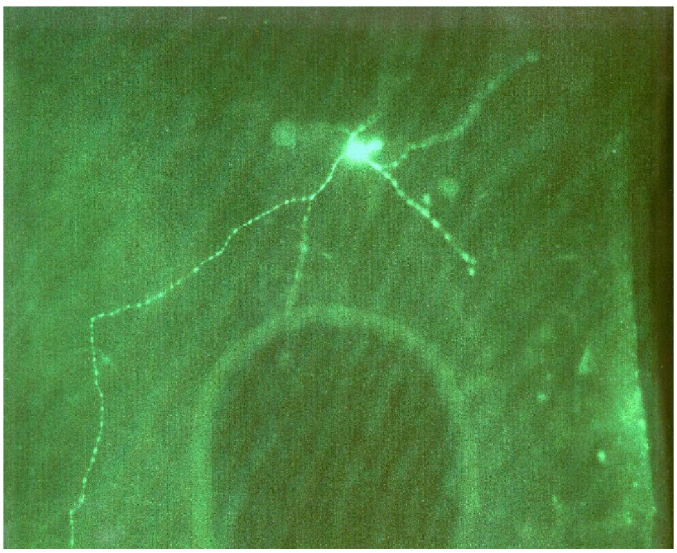

Figure 1 : Investigated neuron in PO/AH of rat

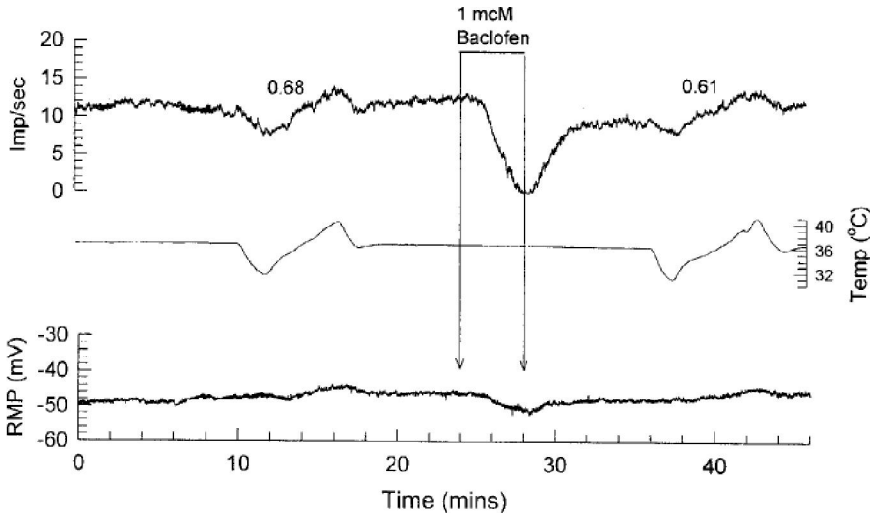

Figure 2 : Effect of the $GABA_B$-receptor agonist baclofen on spontaneous discharge and resting membrane during intracellular recordings

and/or CGP 35348 were applied for 5-10 min and than returned to ACSF.

Only one neuron per slice was tested. After end of experiments the investigated cells were marked with Lucifer Yellow and registered by fluorescent microscope for the Z Stack (Figure 1).

RESULTS

Effects of $GABA_B$-receptor agonist baclofen

Firing rate and resting membrane. The neurons in which the effect of the $GABA_B$-receptor agonist baclofen (1 μM) was tested, reacted with decrease of firing rate, regardless of their type of temperature sensitivity. The decreasing in firing rate during baclofen application was accompanied with statistically significant membrane hyperpolarization in the neurons (Figure 2, Figure 3 and Figure 5).

Original recordings of firing rate (imp/sec), slice temperature (°C) and resting membrane potential (RMP) (mV) from neuron of the medial preoptic area. Superfusion with the $GABA_B$-receptor agonist baclofen (1 μM) decreased firing rate and hyperpolarized resting membrane. Note recovery of the inhibitory and hyperpolarizing effect of baclofen.

The resistance test (MΩ) investigated of the neurons was significantly decreased during baclofen superfusion (Figure 4 and Figure 5).

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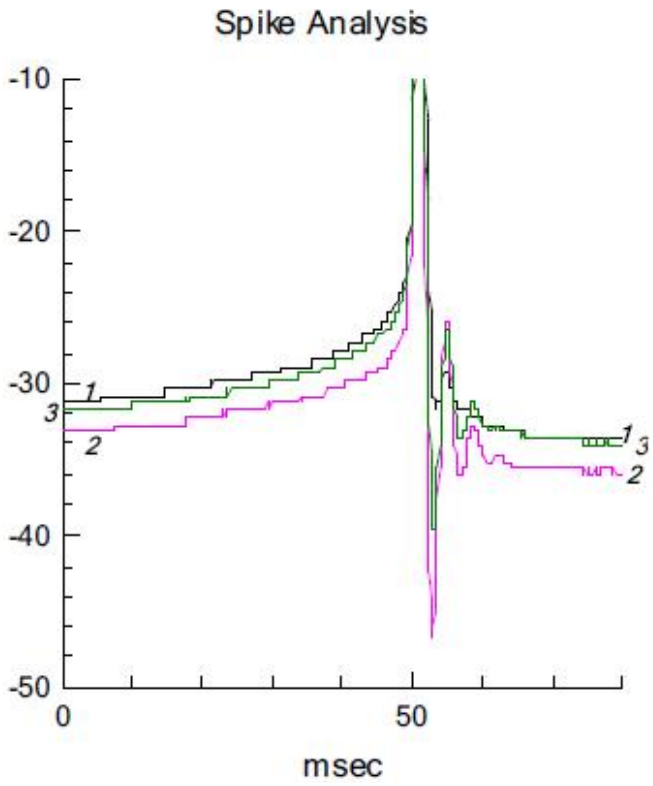

Figure 3 : Spike analysis: *1 - control (before baclofen application); 2- during baclofen superfusion; 3 - control (after baclofen application)*

Recovery in the investigated parameters was observed after wash out the substance (Figure 2 and Figure 5).

Effects of $GABA_B$-receptor antagonist CGP 35348

Firing rate and resting membrane. The neurons in which the effect of the $GABA_B$-receptor antagonist CGP 35348 (100 μM) was tested, reacted with increase of firing rate, regardless of their type of temperature sensitivity. The increasing in firing rate during CGP 35348 application was accompanied with significant membrane depolarization in PO/AH neurons (Figure 4 and Figure 5). The resistance test (MΩ) investigated in both type of neurons was significantly increased during CGP 35348 superfusion (Figure 4 and Figure 5). The effects of both $GABA_B$-receptor antagonist CGP 35348 and $GABA_B$-receptor agonist baclofen were abolished when applied simultaneously.

Original recordings of firing rate (imp/sec), slice temperature (°C) and resting membrane potential (RMP) (mV) from neuron of the medial preoptic area. Superfusion with the $GABA_B$-receptor agonist baclofen (1 μM) decreased firing rate and hyperpolarized resting membrane. Superfusion with the $GABA_B$-receptor antagonist CGP 35348 (100 μM) increased firing rate and derpolarized resting membrane. Neither effects of baclofen nor of CGP 35348 occurred when applied simultaneously. Not changes of the resistance test (RES210) investigated during application of baclofen, CGP 35348 or both baclofen and CGP 35348.

Average data (means±S.E.M.); control (before superfusion of substance), effect (during superfusion), recovery (after superfusion); num-

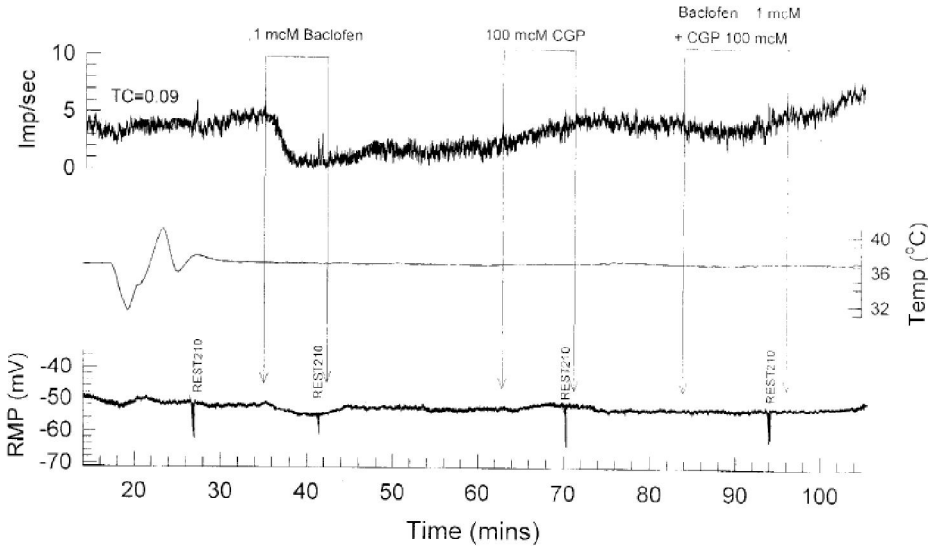

Figure 4 : Effect of the $GABA_B$-receptor antagonist CGP 35348 on spontaneous discharge and resting membrane: $GABA_B$ receptor agonist and antagonist interaction

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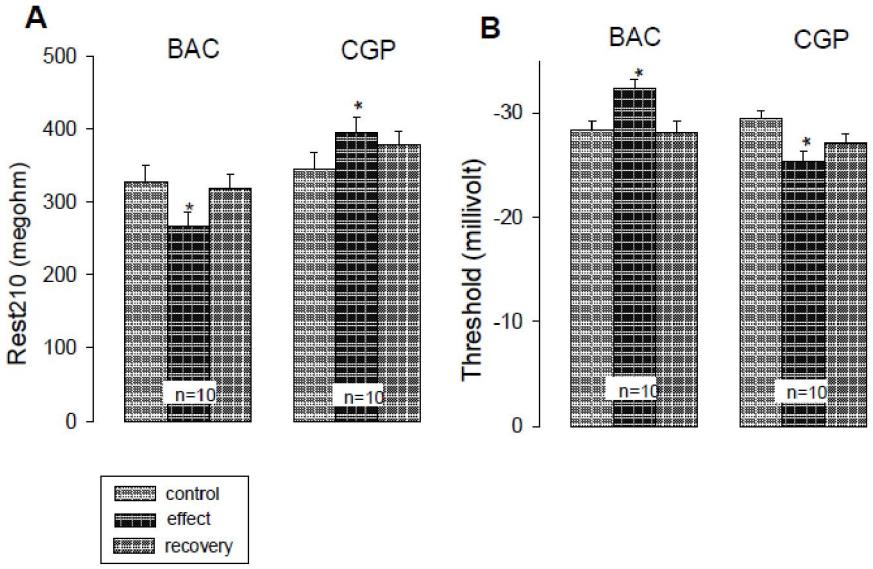

Figure 5 : Effects of the $GABA_B$-receptor agonist baclofen (BAC) (1 µM) and $GABA_B$-receptor antagonist CGP 35348 (CGP) (100 µM) on resistance test (Rest210) (MΩ) (A) and spike analyses thresholds (mV) (B) of PO/AH neurons

ber (n) of recorded PO/AH neurons indicated in the middle columns.

*Significant values: *P<0.05 (comparison with the control period before superfusion of substance).*

DISCUSSION

Previous studies using extracellular recordings of the neurons in rat hypothalamus have shown that firing rate of the majority temperature-sensitive and temperature-insensitive neurons was inhibited by $GABA_A$ (muscimol) and $GABA_B$ (baclofen) agonists. Application of the respective $GABA_A$ and $GABA_B$ antagonists shows the opposite effect. However, the temperature sensitivity of rat PO/AH neurons was only changed by ligands of $GABA_B$-receptor and this effect has been restricted to the warm-sensitive neurons[12].

The present results are step forward, investigating the effects of $GABA_B$ -receptor agonist and antagonist on PO/AH neurons using whole-cell patch-clamp recordings. The present study shows decrease in firing rate during application of $GABA_B$-receptor agonist baclofen which was accompanied with significant membrane hyperpolarization and decrease of input resistances. The firing rate was increased by $GABA_B$-receptor antagonist CGP 35348 and this was accompanied with membrane depolarization and increase of input resistances. Our previous investigations of the effect of baclofen on rat PO/AH neurons by whole-cell patch clamp recordings are in accordance with these findings[13].

There are different investigations to explain the neuronal mechanisms of $GABA_B$ receptors. Activation of $GABA_B$-receptors by GABA or baclofen is mediated by a G-protein and causes a decrease in Ca^{2+}-condactance[14] or an activation of a potential- or Ca^{2+}-dependent K^+-conductance[15]. On postsynaptic level the $GABA_B$ receptor-mediated increase in K^+-conductance generates fast and slow IPSPs. This mechanism can lead to a hyperpolarization often observed in neurons of different brain areas and is probably the main reason for the inhibition of neuronal activity. In the present study we demonstrate significant decrease in firing rate accompanied with hyperpolarization of warm-sensitive, as well as temperature-insensitive PO/AH neurons. The hypothalamic neurons endogenously express to a varying degree to an after hyperpolarization, an inward rectification with an inward current and K^+-current. Such intrinsic and transmitter activated conductance likely serve as important determinants of firing patterns of hypothalamic neurons. In the hypothalamic slices of guinea pigs, with the bath application of $GABA_B$-receptor agonist baclofen the hypothalamic neurons respond with a membrane hyperpolariza-

tion or an outward current[16]. Majority of the neurons tested with $GABA_B$-agonist baclofen responded with membrane hyperpolarization or an outward current[17]. The terminals of both inhibitory (GABAergic) and excitatory (glutamergic) afferents are generally subjected to modulation by presynaptic $GABA_B$-receptors and this modulation is potentially directed to inhibitory inputs[18].

In the present study, co-application of $GABA_B$-receptor agonist and antagonist removed the effects of both agonist and antagonist. The findings observed were abolished when baclofen and CGP 35348 were applied simultaneously. Neither effects of baclofen nor effects of CGP 35348 were found. This is an evidence for specific $GABA_B$ mechanisms in the modulation of neuronal response characteristics in PO/AH of rats. The neuronal network in the anterior hypothalamus appears to be under tonic control of continuously released GABA, since the $GABA_B$-receptor antagonist CGP 35348 influences the response characteristics of rat PO/AH neurons.

The data presented are step of understanding the complicated mechanisms of action of GABA on the level of central temperature controller – the neurons of PO/AH.

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