

Characteristics of GABA Receptors on the Ocellar L-Neurons of American Cockroach *Periplaneta americana*

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Abstract

The ocellar L-neurons of cockroach *Periplaneta americana* were used in the present study as model systems to investigate the pharmacological properties of the GABA receptors. To do so, a glass microelectrode was impaled into the axon of the L-neurons to record the membrane potential intracellularly and to monitor membrane response to GABA treatment and cercal stimulation by air puff. The traditional GABA and their receptor agonists were introduced through perfusion and/or iontophoresis to monitor their effects on the L-neurons. The GABA receptor antagonists were administered by perfusion to examine if the response of the L-neurons to GABA and/or cercal stimulation was changed. The results revealed that administration of GABA, muscimol and imidazole acetic acid, two GABA_A agonists, produced depolarization on the L-neurons. However, treatment of 3-APS and guanidine acetic acid, another two GABA_A agonists, evoked hyperpolarization on the L-neurons. Among those tested antagonists, only picrotoxin, GABA_A antagonist, antagonize the depolarization induced by GABA and/or cercal stimulation. More interestingly, administration of strychnine, glycine receptor antagonist, largely attenuated the depolarization response of the L-neurons to cercal stimulation. This attenuation caused by strychnine was even stronger than that initiated by varied GABA antagonists. In addition, phaclofen, a GABA_B receptor antagonist, showed no antagonistic effect. These results strongly suggest that the characteristics of GABA receptors of the ocellar L-neurons may differ from those in vertebrates. It may be more likely to be a novel GABA receptor.

Key Words: *Periplaneta americana*, ocellar L-neurons, GABA receptors, intracellular recording, iontophoresis

Introduction

There are two compound eyes and two ocelli in the adult American cockroach. Each ocellus consists of approximately 10,000 photoreceptors. Data from cobalt staining reveals that each ocellus in adult cockroach has four L-neurons which are indeed the large ocellar second-order neurons (7). The size of the axons of an L-neuron is about 10-15 μm in diameter and can be easily impaled with a glass microelectrode for intracellular recording. Results from intracellular recording performed on the axon of the L-neurons

have demonstrated that the resting membrane potential is around -55 to -65 mV. After dark adaptation, the L-neurons are hyperpolarized in response to ocellar illumination while depolarized to cercal stimulation. The amplitude of the response is dependent on the intensity of illumination and cercal stimulation, with a maximum of approximately 20 mV (12, 13, 16) and 5 mV (13), respectively. This hyperpolarization or depolarization response is usually maintained for at least 30 min. However, mechanism for the hyperpolarization and/or depolarization is still remained to be determined. We have previously reported that

Table 1. Composition of the experimental salines (concentration in mM, pH = 7.2)

Chemicals	Normal Saline	Na ⁺ -free Saline	K ⁺ -free Saline	High-K ⁺ Saline	Cl ⁻ -free Saline
NaCl	214.0	---	217.1	86.1	---
KCl	3.1	3.1	---	31.0	---
CaCl ₂	1.8	1.8	1.8	1.8	---
Tris-HCl	10.0	10.0	10.0	10.0	10.0
Na gluconate	---	---	---	---	214.0
K gluconate	---	---	---	---	3.1
Ca gluconate	---	---	---	---	1.8
Choline chloride	---	214.0	---	---	—

there are at least four functionally different receptors on the ocellar L-neurons in the American cockroach (13), and further demonstrated that GABA is one of the putative neurotransmitters located on the efferent axon, which is also called S-neurons in the locust (1) and cockroach ocellus. However, we still know nothing about the characteristics of this GABA receptor, specifically, in cockroach L-neurons. It has been shown that GABA administration elicit excitatory responses in the stomatogastric ganglion of the crab *Cancer borealis*. This excitatory effect is very much different from the GABA systems in the vertebrates. Specifically, the insect GABA receptors are not inhibited by bicuculline and phaclofen, the most GABA antagonist used in vertebrate GABA receptors (20).

The same results have also been observed in GABA receptors of cultured neurons in cockroach, *Periplaneta americana*. In this regard, Aydar and Beadle (2) has found that GABA-induced responses are effectively blocked by perfusion of picrotoxin, but not completely affected by perfusion of bicuculline at a concentration of 10⁻⁴ M and/or 10⁻⁵ M. They conclude that insect GABA receptors may have novel pharmacological properties different from any kinds of vertebrate GABA receptors. These results raise a question whether GABA receptors in cockroach L-neurons differ from those in vertebrates? What are the pharmacological characteristics of the GABA receptors in cockroach L-neurons? However, there is no such reports to answer this question.

In this study, we used the GABA receptors by acting on the ocellar L-neurons of American cockroach as a model to examine the pharmacological properties of the GABA receptor. The data obtained confirmed that the GABA receptors located on the L-neurons of cockroach differed from that of the vertebrates.

Materials and Methods

Animal Preparation

The cockroaches were male American cockroach

Periplaneta americana which were kept at 25 ± 2°C in a 12 h light/12 h dark photoperiodic regime for at least two weeks. They were reared on running water and chicken diet (TaiSugar company, Taiwan). During experiment, the cockroach was put into a small box, which was maintained at low temperature (0°C) for anesthesia. Usually, it took 5-10 min to immobilize the cockroach. It was then mounted on the stand. Its head was immersed into a 2 ml chamber, which was perfused with normal saline or saline containing freedom from Cl, Na or K ions (Table 1). The head was partially dissected to expose the brain and ocellar nerve. The connective tissues around the ocellar nerves were then softened with 1% protease (Sigma protease XIV, Sigma, St. Louis, MO, USA) to facilitate the impalement of glass microelectrode. A small piece of platinum was placed in the chamber as an indifference electrode. For cercal stimulation, an air puff guide of 2 mm in diameter was set 10 mm apart from the right cercus. The air was derived from a compressor and was delivered toward the cercus through the silicon tubes by an electric valve. The airflow of the puff was controlled at a rate between 3 and 4 m/sec through a flow meter (13).

Intracellular Recording

A microelectrode with a resistance of 70-100 MΩ was filled with 2.5 M potassium acetate was then impaled into the axon of the L-neuron for intracellular recording. Signals of the membrane potentials picked up by this microelectrode were amplified with a DC-amplifier (Nikon Kohden, MEZ-8301, Tokyo, Japan) and displayed on an oscilloscope (Gould 1604, LLFORD, Essex, UK) as was described in our previous reports (11-13). Two cerci were puffed simultaneously with a stimulator (Nihon Kohden, SEN 7203, Nihon Kohden, Tokyo, Japan) to control the air source once every minute with a duration of 0.5 sec. Membrane depolarization induced by reflex stimulation of air puff delivery to the cercus was displayed on an

Table 2. Effects of GABA, its agonists and antagonists on the membrane potential and cercal responses of L-neurons

Chemicals	Specificity [#]	Conc. (mM)	Membrane Potential [@]	Cercal Response
GABA	A+, B+	0.1	depol(*)	reduced
Muscimol	A+	0.5	depol *	reduced
Imidazole acetic acid	A+	1.0	depol	reduced
3-APS	A+	0.1	hyper	reduced
Guanidine acetic acid	A+	0.1	hyper	reduced
THIP	A+	0.1	---(*)	---
Picrotoxin	A-	0.1	---	inhibited
Strychnine	A-	0.05	---	inhibited
Bicuculline	A-	0.1	---	---
Bicuculline methiodide	A-	0.1	---	---
Nipecotnic acid	A-	0.1	---	---
Baclofen	B+	0.1	---(*)	---
Phaclofen	B-	0.1	---	---

[#]Vertebrate GABA receptors have GABA_A (A) and GABA_B (B); + denotes agonist; - is antagonist.

[@] shows different treatments: *, iontophoresis; (*), both perfusion and iontophoresis; ---, ineffective;

3-APS: 3-aminopropane sulphonic acid;

THIP: 4,5,6-tetrahydroisoxazolo(4,5,-c)pyridin-3-ol.

oscilloscope. All data were electronically stored (DX-900, PCM, Toshiba, Tokyo, Japan) and simultaneously recorded on chart paper (8k21, NEC SAN-EI, Tokyo, Japan).

Iontophoresis

To induce depolarization occurred in the L-neurons, GABA (1.0 M, pH = 3.5) was applied through a double-barrelled micropipette by iontophoresis. To achieve this aim, GABA solution was prepared and adjusted the pH value to an extent of 3.5 so that the GABA solution charged positively. With this adjustment, GABA could be administered iontophoretically. One of the double-barrelled micropipette was filled with GABA solution and the other was filled with 0.2 M NaCl solution for control injection. The micropipette was positioned by using a micromanipulator to localize within the ocellar cup. A pulse of GABA was then ejected by a current of 10 nA with a duration of 5 sec (BH-2, Medical System, New York, USA). Control injection of 0.2 M NaCl or saline solution was randomly applied with the same manner as that of GABA.

Perfusion

To prevent the effects of synaptic transmission on efferent stimulation or application of chemicals, saline with Ca⁺⁺-free and Co⁺⁺ was perfused only or with test chemicals. In some other studies, experimental salines (Table 1) without specific ions

were perfused to test the ionic effects on the response of GABA receptors to varied stimuli.

To evaluate the possibility that GABA receptor on the L-neurons might mediate the depolarization response induced by cercal stimulation, ocellar neuropil was perfused with varied GABA receptor antagonists (all purchased from Sigma). Chemicals were first dissolved in saline (pH = 7.2) to make a solution with a concentration of 1.0×10^{-3} M as stock and stored in a deep freezer. This stock solution was then diluted during experiment to a concentration, which produced the minimum effective response occurred in the neuron and was defined as threshold concentration (Table 2). The perfusion rate was set at 20 ml/min. Experiments were all carried out at room temperature ($22 \pm 2^\circ\text{C}$).

Results

Responses of L-Neurons to Cercal Stimulation and GABA

The ocellar L-neuron was depolarized in response to cercal stimulation (Figs. 1A, 3 and 4-6). The amplitude of the depolarization was about 5 mV. Iontophoretical application of GABA (20 nA, 5 sec) into the ocellar cup also produced depolarization occurred on the same L-neuron with a maximum response of 4-5 mV (Figs. 1B, 2, 5 and 7) which was similar to the response to cercal stimulation.

Perfusion of synaptic blockade, CoCl₂ (10 mM) on the ocellar neuropil totally abolished the depolarization

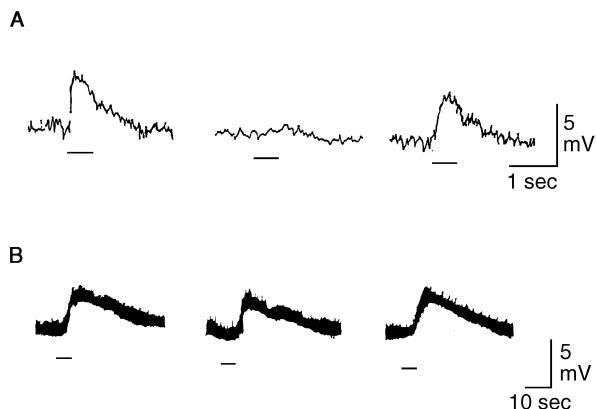


Fig. 1. Effects of perfusion of Ca^{++} -free saline with 10 mM CoCl_2 upon the responses of ocellar L-neurons to cercal stimulation and iontophoresis of GABA.

A. The L-neuron was depolarized first by air puff onto the cercus for 0.5 sec (horizontal bar, cercal stimulation) to make sure that the microelectrode was positioned correctly inside the L-neuron (left panel). Cercal stimulation producing depolarization in the L-neuron was abolished by a perfusion of saline containing Ca^{++} -free and 10 mM CoCl_2 into the ocellar cup (middle panel) and recovered after 25 min-washing (right panel). B. After making sure the position of the microelectrode impaled inside the L-neurons by cercal stimulation, GABA was then applied iontophoretically to the ocellar cup at a 20 nA current and a 5 sec duration (horizontal bar) to induce depolarization of the L-neuron (left panel). This depolarization caused by GABA iontophoresis was persisted no matter with (middle panel) or without (right panel) perfusion of Ca^{++} -free saline plus 10 mM CoCl_2 into the ocellar cup. Data in the middle panel was taken from perfusion for 20 min.

response to cercal stimulation (Fig. 1A), suggesting that chemical synapse might be involved and that Ca^{++} might play a role in this synaptic transmission. This is due to the Co^{++} competition with the Ca^{++} at active sites of the presynaptic terminals and in turn synaptic transmission was blocked.

Following improvement of the chemical synaptic transmission, the GABA solution was then iontophoretically introduced into the ocellar neuropil to examine if this chemical transmission was GABA-mediated. To do so, GABA was iontophoretically applied to the ocellar cup to evoke depolarization of the L-neurons (Fig. 1B, left). This depolarization response seen in the L-neurons was persisted no matter CoCl_2 was present (Fig. 1B, middle) or absent (Fig. 1B, right). This persistence of GABA-induced depolarization after blockade of synaptic transmission with CoCl_2 suggested that GABA receptors were probably presented on the L-neurons. Thus, both cercal stimulation and iontophoresis of GABA into the ocellar cup induced occurrence of depolarization in the same

L-neuron. The only difference was that depolarization induced by cercal stimulation was easily blocked by synaptic blockade while GABA application was not. These results strongly indicated that GABA might act on the postsynaptic neuron, the L-neurons, while cercal stimulation might produce a reflex excitation to the L-neuron *via* chemical synaptic transmission. However, GABA-induced depolarization in the L-neurons was not easily to be recorded due to the spatial organization. Therefore, depolarization responses in most of the experiments were only induced by a duration of 5 sec for the study of GABA iontophoresis (Figs. 1, 2, 5 and 7), and also for the cercal stimulation (Figs. 1, 3 and 4-6).

Effects of GABA Receptor Agonists and Antagonists upon L-Neurons

There are two types of pharmacologically distinctive GABA receptors, subtypes A and B, in the vertebrate neurons. To characterize the GABA receptors and to determine which type of GABA receptors might locate on the cockroach L-neurons, the following experiments were performed.

(A) Comparison of the Potency between GABA and Muscimol

Iontophoresis of GABA and/or muscimol, a GABA_A receptor agonist, into the ocellar cup was performed to depolarize the membrane potential of the L-neurons (Fig. 2). By changing the pH value of the solution, GABA and muscimol was positively charged so that they could be delivered through iontophoresis to the ocellar neuropil. To compare the potency of muscimol with that of GABA, muscimol and GABA were alternately applied by iontophoretic current delivery to the double-barreled micropipette, one filled with GABA and the other containing muscimol. The extent of depolarization of the L-neuron caused by GABA was increased in a dose-dependent manner from 5 to 25 nA and was then saturated when the current was raised above 30 nA (Fig. 2A). However, membrane depolarization caused by muscimol was not detected until 30 nA. There was a dose-dependent response manner above 30 nA (Fig. 2B).

(B) Interaction of GABA and/or Agonist with Cercal Stimulation

The ocellar neuropil was perfused with saline containing 0.1 mM GABA to produce gradual depolarization of the L-neuron. The membrane potential was returned to the control level after cessation of GABA perfusion (Fig. 3A). During the period of perfusion, cercal stimulation still produced

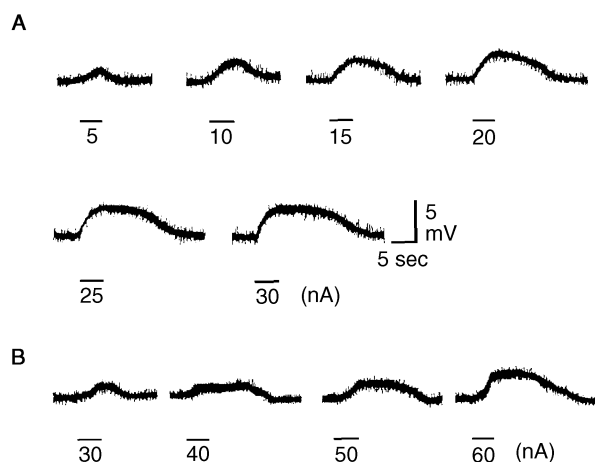


Fig. 2. Responses of the ocellar L-neurons to iontophoresis of GABA and muscimol.

A. The L-neuron was depolarized by the iontophoresis of GABA. The amplitude of depolarization with GABA administration was increased in proportional to the increase in the injected current (nA). The numbers at the bottom on each panel indicated the current intensity applied. The duration of injections was 5 sec (horizontal bar). B. The L-neuron was depolarized by the iontophoresis of muscimol (a GABA_A agonist). The amplitude of depolarization was in proportional to the increase in the injected current applied to muscimol. The duration and current intensity were expressed by the horizontal bar (5 sec) and the number underlined.

depolarization of the L-neuron. However, the degree of the depolarization caused by cercal stimulation was reduced following the gradual increase of the membrane depolarization evoked by GABA perfusion (Fig. 3, A and B). This decrease of response, in terms of depolarization, to cercal stimulation was recovered when GABA perfusion was ceased (Fig. 3A) and thus a full response of depolarization was obtained (Fig. 3, A and B). It seems that the depolarization in response to cercal stimulation was superimposed on the membrane changes induced by GABA perfusion. Thus, sum of the membrane depolarization evoked by both cercal stimulation and GABA perfusion was about 5 mV as was shown in Fig. 3A. Perfusion of imidazole acetic acid, a GABA_A receptor agonist, produced a similar depolarization response occurred upon the L-neuron (data not shown) as did as the application of GABA. However, perfusion of 3-APS (3-aminopropane sulphonic acid) with a concentration of 0.1 mM, another GABA_A receptor agonists, induced hyperpolarization on the L-neurons rather excitation (Fig. 4). Under this hyperpolarization caused by 3-APS, membrane depolarization in response to cercal stimulation was remained by restrained to a situation very similar to that caused by GABA. Perfusion of

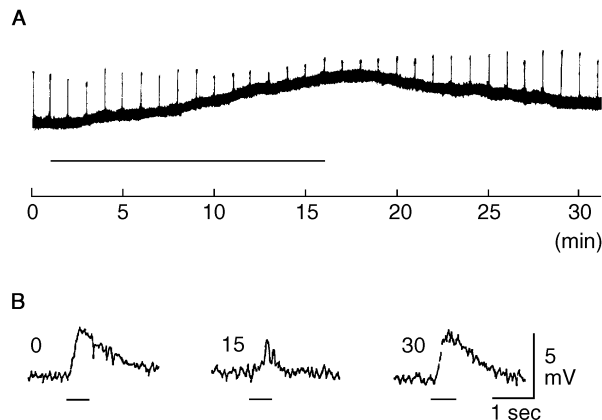


Fig. 3. Effects of GABA perfusion on depolarization response of the L-neuron to cercal stimulation.

A. The cercus was stimulated by air puff to produce depolarization showing gradual decrease in amplitude following the perfusion of low concentration of GABA solution (0.1 mM) (horizontal bar). The perfusion of GABA gradually depolarized the L-neuron and reduced the response to air puff stimulation applied to the cercus. Cessation of GABA perfusion made the membrane potential of the L-neuron back to control level and restored also the ability of the L-neuron in response to cercal stimulation again. B. Three recordings of depolarization response to cercal stimulation which was taken from the tracing in panel A and enlarged temporally to show the potential profile. The amplitude of the depolarization caused by cercal stimulation was much reduced after 15 min GABA perfusion compared with the one before (0 sec) and the other one 30 min after cessation of GABA perfusion,

guanidine acetic acid, another GABA_A receptor agonist used in the present study, also produced hyperpolarization on the L-neuron similar to that caused by 3-APS (data not shown). In contrast, microinjection of THIP (4,5,6-tetrahydroisoxazolo (4,5,-c)pyridin-3-ol), a GABA_A receptor agonist, and perfusion of baclofen, a GABA_B receptor agonist, (all 0.1 mM), failed to induce depolarization on the L-neurons (Table 2).

(C) Effect of GABA Antagonist

Picrotoxin, bicuculline, bicuculline methiodide, and nipecotic acid are all traditional GABA_A receptor antagonists. Among them, only picrotoxin (0.1 mM) effectively suppressed both cercal stimulation- and GABA-induced depolarization as was shown in Fig. 5. Application of phaclofen, a GABA_B receptor antagonist, produced no effects on the cercal stimulation-induced depolarization at the concentration of 0.1 mM (Table 2). These results strongly suggest that the pharmacological characteristics of GABA receptor were

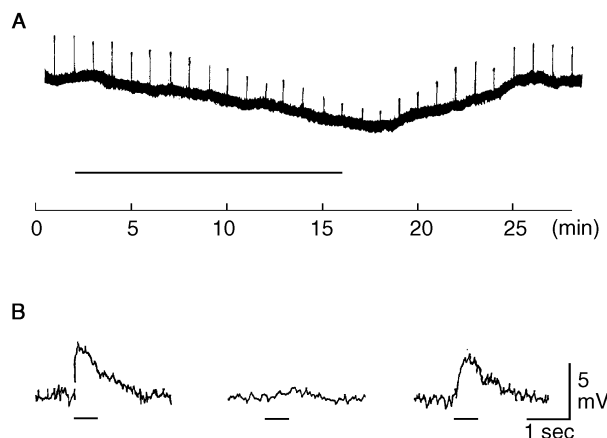


Fig. 4. Effects of GABA agonists on the L-neurons. A. Perfusion (horizontal bar) of 3-aminopropane sulphonic acid (3-APS) with a concentration of 0.1 mM, a GABA_A agonist, onto the ocellar neuropil reversibly reduced the amplitude of cercal responses which was similar to that caused by GABA but hyperpolarized the membrane potential of the L-neurons instead of depolarization. B. Three recordings of a depolarization response to cercal stimulation taken from panel A were temporally enlarged to display the profile of the membrane potential. It is clear that amplitude of membrane depolarization in response to cercal stimulation was much reduced after 15 min perfusion of 3-APS compared with the response before (panel B, middle) and 30 min after cessation of perfusion (panel B, right).

shown on the ocellar L-neurons of the American cockroaches and clearly distinguished from those of the traditional GABA receptors seen in the vertebrate neurons.

Effects of Glycine Antagonists on the L-Neurons

Perfusion of strychnine, a glycine receptor antagonist, with a relatively low concentration was found to inhibit the response induced by cercal stimulation (Fig. 6, Table 2). The minimum concentration for strychnine to suppress cercal stimulation inducing depolarization was less than 0.05 mM. Compared with the action of GABA_A and GABA_B antagonist, strychnine was more effective to suppress the depolarization response than any kinds of GABA_A or GABA_B antagonists used in the present study to block the GABA action (Table 2).

Effects of Chloride-Free Saline on the L-Neurons

Perfusion of chloride-free saline hyperpolarized the L-neurons and largely enhanced the GABA-induced responses of the L-neurons (Fig. 7). However, perfusion of sodium-free saline, potassium-free saline

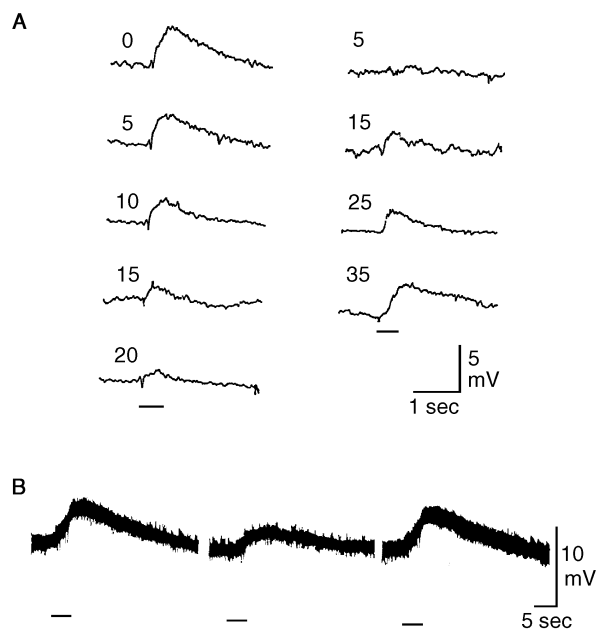


Fig. 5. Effects of picrotoxin on the L-neurons. A. Perfusion of 10⁻⁴ M picrotoxin produced no changes in membrane potential on the L-neurons but effectively inhibited its response to depolarization induced by air puff (panel A, left). Duration for cercal stimulation was 0.5 s (horizontal line on the left panel). The number on the upper left corner of each tracing indicates the time after picrotoxin perfusion. This inhibition of depolarization in response to cercal stimulation was gradually recovered after cessation of picrotoxin perfusion and full response to cercal stimulation was observable at 35 min after ceasing the perfusion. B. Perfusion of the same concentration of picrotoxin (10⁻⁴ M) also antagonized the depolarization of the L-neurons induced by GABA with a similar time course. Thus, depolarization induced by GABA iontophoresis (20 nA with a duration of 5 sec) applied to the ocellar neuropil was antagonized after 20 min perfusion of picrotoxin (panel B, middle). The response of the L-neuron to GABA iontophoresis was totally recovered after washing for 25 min (panel B, right).

and high-potassium saline (Table 1) had no effects on the depolarization of the L-neurons evoked by iontophoretic application of GABA (Table 3). These results strongly suggest that the GABA-induced depolarization on the ocellar L-neurons of American cockroaches might mediate through the increase in the permeability of chloride ion rather than sodium and/or potassium ions.

Discussion

In this study, we used the ocellar L-neurons of American cockroach as a model to investigate the pharmacological characteristics of the GABA

Table 3. Effects of ion compositions on the membrane potential and responses of the L-neurons to GABA

Experimental saline (replaced by)	Membrane potential	GABA-induced response
Na ⁺ -free (choline)	depolarization	---
K ⁺ -free (Na ⁺)	---	---
High-K ⁺	---	---
Cl ⁻ -free (gluconate)	hyperpolarization	enhancement

---: ineffective

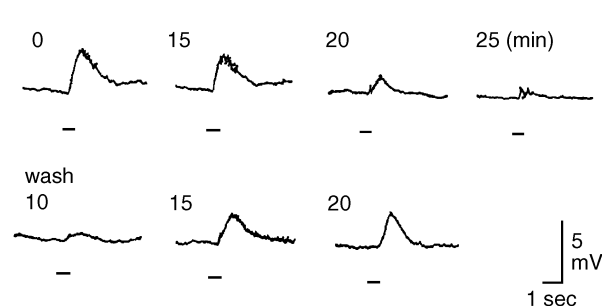


Fig. 6. Effects of strychnine on the L-neurons.

Perfusion of 0.05 mM strychnine, a glycine receptor antagonist, produced no changes in membrane potential in the L-neurons but reduced the depolarization response to air puff delivery to the cercus with a duration of 0.5 sec (upper panel). This inhibition of strychnine was reversible so that the response was fully resumed (lower panel). The number on the upper left corner of each tracing indicates the time of perfusion (upper panel) or after washing (lower panel) during which cercal stimulation was delivered.

receptor. This allowed us to compare with the GABA receptor in vertebrate nervous tissues. Based on our data about the application of GABA and muscimol elicited depolarization responses occurred in the L-neuron, the GABA receptor might have an excitation in nature on the ocellar L-neurons after activation. The GABA-evoked depolarization, which was similar to the response induced by cercal stimulation, were only blocked by picrotoxin but insensitive to bicuculline, bicuculline methiodide and phaclofen. Moreover, depletion of chloride ions from the extracellular medium enhanced the GABA-mediated depolarization. These results strongly suggest that GABA receptor located on the L-neuron of the American cockroach is very similar, but not identical, to that of the GABA_A receptor described in vertebrate nervous tissues. Interestingly, the excitatory response of the L-neurons to cercal stimulation was even more sensitive and efficient to be inhibited by strychnine, a glycine receptor antagonist, *vs.* by any kinds of traditional GABA receptor antagonists.

The GABA receptors in the vertebrates have

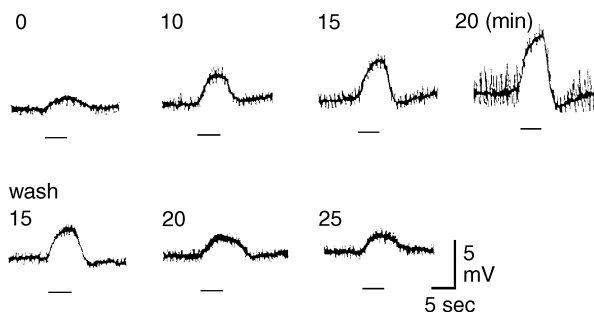


Fig. 7. Effects of perfusion of Cl⁻-free saline on the response of the ocellar L-neurons to iontophoresis of GABA.

Depolarization response of the L-neurons to GABA iontophoresis was largely enhanced by perfusion of saline with Cl⁻-free. This enhancement of depolarization caused by GABA was time-dependent, giving the number in min on the left corner of each tracing, following perfusion of saline containing no Cl ions (upper panel). This excitatory effect of saline without Cl ions was reversible so that the depolarization response to GABA was gradually recovered (lower panel). The GABA injected current was 10 nA with a duration of 5 sec (horizontal bar).

been classified into subtypes A and B based on the pharmacological studies (5). The receptors whose responses can be activated by GABA or muscimol and blocked by bicuculline are classified as GABA_A receptor. In contrast, the receptors whose responses can be activated by GABA or baclofen but blocked by phaclofen and not blocked by bicuculline are named as GABA_B receptor. Generally speaking, GABA_A receptor itself is also an ion channel gating chloride ion and allosterically modulated by barbiturates and benzodiazepines (10). On the contrary, GABA_B receptor regulates K⁺ and Ca⁺⁺ channels through GTP-binding protein and intracellular messenger pathways at the cellular level (4).

Our present results provided information that insect GABA receptor might have differences in nature at least in the L-neurons of American cockroach when compared with the GABA receptors in the vertebrates. This notion is compatible with other reports. Datum *et al.* (8) studied immunocytochemically the compound eye of the fly *Calliphora erythrocephala* and found

that the photoreceptors R7 and the efferent C2 neurons used GABA as a neurotransmitter. Sattelle *et al.*, (14, 17-19) reported that bicuculline-insensitive GABA-operated Cl⁻ channels in the nervous tissue of cockroach but just rare in the vertebrates. Buckingham *et al.*, (6) reported that GABA elicited a dose-dependent increase in membrane conductance in the giant interneurone 2 (GI 2) of the cockroach *Periplaneta americana*. This increase in membrane conductance was not blocked by bicuculline, a GABA_A receptor antagonists of mammals. Bai and Sattelle (3) found that several vertebrate GABA_B receptor agonists produced hyperpolarization of motor neurone Df in the metathoracic ganglion of the cockroach (*Periplaneta americana*). However, baclofen failed to induce any responses on the same neuron. The responses evoked by GABA_B receptor agonists were also insensitive to bicuculline. Aydar and Beadle (2) reported that GABA receptors on cultured cockroach neurons responded to pressure application of GABA and muscimol; however, did not respond in membrane potential or membrane conductance to the application of baclofen even at concentrations as high as 10 mM. Our data and these reports provide evidence that the GABA receptors presented in insects such as the cockroach may differ in characteristics from those of the vertebrates.

This difference in characteristics of the GABA receptor between insect and vertebrate might be due to the conformational structure as was reflected in our present data, showing that the ocellar L-neuron was excited by muscimol and imidazole acetic acid but inhibited by 3-APS and guanidine acetic acid. These four substances are all classified as GABA_A receptor agonist in the vertebrate but presenting opposite action on the ocellar L-neurons. This paradoxical phenomenon may ascribe to the conformational difference of the GABA receptor in the insect *vs.* the vertebrate. Since the depolarization caused by GABA and/or muscimol was enhanced by depletion of extracellular chloride ions, suggesting that insect GABA receptor may be Cl⁻-mediated, in terms of this mechanism as to the chloride permeability, insect GABA receptor may have similarity to that described in the vertebrate. Nevertheless, this may only give explanation for the hyperpolarization induced by 3-APS but not for the action of muscimol. Moreover, depolarization response of the insect GABA receptor was not related to the changes in cations such as Na⁺ and K⁺. This controversy has been observed in the histamine receptor of the American cockroach (13). Unfortunately, the ionic mechanism for this paradox is still ambiguous.

Another point to support this difference in characteristics of the GABA receptors between the insects and vertebrates was the action of strychnine, a glycine receptor antagonist on the GABA receptor. This may be explained that insect GABA receptor

probably contains binding site for the glycine or strychnine. This property is totally different from the GABA receptors in the vertebrate and has been reported by Hamill *et al.*, (9). They argue that there may be a possible combination of glycine and GABA receptor. Taking another point of view, our data may provide a clear evidence to demonstrate the dissimilarity of GABA receptor between the insect and vertebrate. More interesting was that strychnine was the most potency among those GABA antagonists used in the present study, showing that the order of potency was as following: strychnine > picrotoxin (GABA_A) > bicuculline, bicuculline methiodide, nipecotic acid (GABA_A), phaclofen (GABA_B).

Finally, the ocellar L-neurons in cockroach may play a role in the sensory information transmission (12, 15, 16). In this regard, GABA receptor located on the L-neurons might be one of the mediators. Alternatively, the result showing that excitation caused by cercal stimulation was reversibly attenuated by picrotoxin, a GABA_A receptor antagonist might have another implication that this sensory signal of air-puffing stimulation was probably modulated by an internal GABAergic inputs. This speculation was based on two of our observations. First, perfusion of low concentration of GABA attenuated the extent of depolarization of the L-neurons in response to cercal stimulation (Fig. 5). Sum of the depolarization degree evoked by GABA administration and cercal stimulation seems to be constant, suggesting that these two inputs might have shared the same excitatory mechanism. Second, perfusion of other GABA_A receptor agonist such as 3-APS produced also attenuation of the depolarization response of the L-neuron to cercal stimulation although hyperpolarization was occurred. However, our present data did not provide any evidence to elucidate this putative internal neural pathway.

In summary, our results suggest that the characteristics of the GABA receptor on the ocellar L-neurons of cockroach is different from those has been described in the vertebrate neurons. This dissimilarity may ascribe to the receptor itself in nature. It may be more likely to be a novel GABA receptor.

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