Acta Biologica Hungarica 53 (1–2), pp. 105–123 (2002)

CADMIUM IONS MODULATE GABA INDUCED CURRENTS IN MOLLUSCAN NEURONS*

G. MOLNÁR, J. GYŐRI, J. SALÁNKI**, KATALIN S.-RÓZSA

Balaton Limnological Research Institute of the Hungarian Academy of Sciences,
H-8237 Tihany, Hungary

(Received: September 30, 2001; accepted: November 17, 2001)

The effect of Cd^{2+}, as one of the most widespread toxic environmental pollutants, was studied on γ-aminobutyric acid (GABA) evoked responses of identified neurons in the central nervous system of the pond snail, *Lymnaea stagnalis* L. (Gastropoda). In the experiments, the modulation of the action of GABA both on neuronal activity (current clamp recording) and on the a GABA activated membrane Cl^--current (voltage clamp studies) has been shown. It was found that:

1. GABA could evoked three different various types of response in GABA sensitive neurons: i) hyperpolarization with strong inhibition of ongoing spike activity, ii) short depolarization with an increase of spike the activity, iii) biphasic respone with a short excitation followed by a more prolonged long inhibition.

2. In low-Cl^- solution the inhibitory action of GABA was reduced or eliminated, but the excitatory one was not or only moderately affected.

3. $CdCl_2$ inhibited the GABA evoked hyperpolarization, but left intact or only slightly reduced the excitation evoked by GABA.

4. The inward Cl^--current evoked by GABA at a –75 mV holding potential was slightly augmented in the presence of 1 μmol/l Cd^{2+}, but was reduced or blocked at higher cadmium concentrations. The effect of Cd^{2+} was concentration and time dependent.

5. Parallel with reducing the GABA evoked current, cadmium increased both the time to peak and the half inactivation time of the current.

6. $CdCl_2$ alone, in 50 μmol/l concentration, induced a 1–2 nA inward current.

The blocking effect of cadmium on GABA activated inhibitory processes can be an important component of the neuro-toxic effects of this heavy metal ion.

Keywords: GABA – Cd^{2+}, Cl^--current – *Lymnaea stagnalis* L.

INTRODUCTION

Among anthropogenic pollutants affecting regulatory mechanisms in animals (and humans) heavy metals represent a special group, due to their persistency, widespread distribution and steadily growing increasing concentration in the environment and

*Dedicated to Professor József Hámori on the occasion of his 70th birthday.

**Corresponding author; e-mail: salanki@tres.blki.hu

easy uptake by living organisms through the food chain. Toxic metals such as mercury, lead, cadmium, tin and others have an effect on all living things. They can cause various health disorders in humans as a result of binding to membranes, interacting with molecular processes and thereby causing alterations in basic regulatory mechanisms, among others in the regulation of basic neuronal functions [5]. One of the fundamental structures and physiological processes that appear to be frequently targeted by heavy metals is the neuronal cell membrane and its permeability properties, which are involved in neural regulation through their sensitivity to neurotransmitters [17, 32].

Some of the heavy metals are able to induce an inward current directly or by affecting intracellular mechanisms. Weinreich and Wonderlin [35] found that Cu^{2+}-ions induce an inward current in *Aplysia* neurons by activating Na-channels, while Oortgiesen et al. [23] reported on special currents activated by Al^{3+}-, Cd^{2+}- and Pb^{2+}-ions in cultured mouse neuroblastoma cells. Ag-ions have been shown to activate non-specific cationic channels in *Helix* neurons by increasing cytoplasmic free Ca^{2+}-ion concentration [10]. Also, Hg^{2+}-ions evoked an inward current in neurons of the rat dorsal root ganglion [2] and in the CNS of *Helix* [25].

As far as the effects of metal pollution on neuronal function are concerned, the modulation of the synaptic transmission and of transmitter activated currents by heavy metals is of primary importance. Mainly inhibitory effects were recorded in the presence of heavy metals, however, in some cases enhancement of synaptic transmission was also observed. In molluscan neurons, the inhibitory and/or modulatory effects of Hg^{2+} and Cd^{2+} were described on the potential generation evoked by ACh, DA and 5-HT [30, 32], and also the selective, channel specific influence of Pb^{2+} has been demonstrated on the early and delayed membrane effects of ACh and 5-HT [33].

The inhibition and potentiation of transmitter activated currents by heavy metals are metal and channel specific. The ACh evoked K^+-current was inhibited, however, the Na^+- and Cl^--dependent ACh induced currents were potentiated by mercury ions in *Aplysia* neurons [11]. Lead ions reduced NMDA-activated Ca^{2+}-currents in rat [4], but in the snail, *Lymnaea*, lead enhanced the glutamate activated Cl^--current [28]. GABA induced Cl^--current was augmented by mercury and lanthanium, but was suppressed by copper, cadmium, lead and some other polyvalent cations in neurons of rat dorsal root ganglia [19, 21]. GABA-evoked Cl^--current was potentiated by low concentrations of mercury (0.01–1 μmol/l) also on *Lymnaea* neurons, while higher Hg^{2+}-concentrations caused depression [25].

Recently, as a continuation of our earlier investigations concerning the influence of environmental pollutants on neural regulation in invertebrates, the effect of cadmium on GABA induced membrane events was studied. Our aim was to clarify the effect of cadmium on GABA evoked potential generation, and on the GABA activated Cl^--current in identified, GABA sensitive neurons of the snail *Lymnaea stagnalis* L.

MATERIALS AND METHODS

Animals

The experiments were performed on adult specimens of the pond snail, *Lymnaea stagnalis* L. (Pulmonata, Basommatophora) collected from their natural habitat (Balaton-Minor), and kept in aquarium for weeks before use. For the experiments in winter, the animals were collected in October and survived well for months in slowly running natural lake water, at a temperature of 15–20 °C. The animals were fed with fresh lettuce *ad libitum*. The total length of the animals' shells varied between 40 and 50 mm.

Preparation of the ganglia

After taking out the animal from the shell, the soft body was dissected and the ganglionic mass was separated. Following mechanical removal of the thick connective tissue sheath covering the ganglia, an enzymatic treatment was performed by protease E (type XIV, Sigma) dissolved in physiological solution for 10 min at room temperature. This softened the thin connective tissue sheath and aided the impalement of the selected neuron with microelectrodes. After washing out the protease, the ganglia were kept in a refrigerator at 4 °C at least for 2 hours in *Lymnaea* physiological saline. For the measurements identified neurons, located in the suboesophageal ganglionic ring, were used (Fig. 1). Cells are marked and named according to the map published by Winlow and Benjamin [36], and Rubakhin et al. [26]. Experiments were performed at room temperature (21–24 °C).

Solutions

The standard physiological solution (SPS) used in the experiments contained (in mmol/l) NaCl 40, KCl 1.7, $CaCl_2$ 4, $MgCl_2$ 1.5, glucose 10, Tris-HCl 10, Tris-Cl 10; pH was adjusted to 7.4 by using NaOH. In low-chloride saline NaCl was substituted by Na-acetate (44 mmol/l) and instead of Tris, as buffer, Hepes (10 mmol/l) was used. All chemicals were prepared in SPS. The following substances were used in the experiments: γ-aminobutyric acid, bicuculline, picrotoxin, – all from Sigma, glucose, Hepes and Tris from Sigma, $CdCl_2$ and other inorganic salts from Reanal.

Experimental protocol

Two types of experiment were conducted:

(a) Resting and action potentials were recorded by using 2.5 mol/l KCl-filled microelectrodes from a variety of identified neuron types (RPaD1, RPaD2, VD 2–3, VV2, VDC and J), located on the dorsal surface of the visceral and right parietal gan-

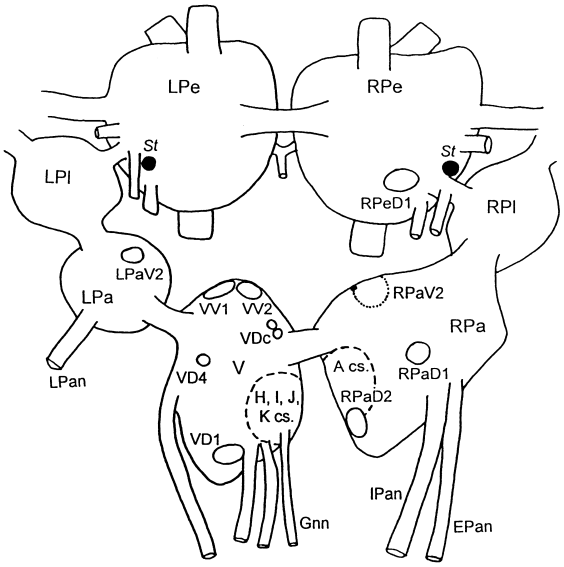

Fig. 1. Schematic representation of the identified neurons used in the experiments in the central nervous system of *Lymnaea stagnalis* L. LPe – left pedal ganglion, RPe – right pedal ganglion, LPl and RPl – left and right pleural ganglia, LPa and RPa – left and right parietal ganglia, V – visceral ganglion, St – statocysta, cs – cluster, IPan – right internal parietal nerve (i.p.n.). Letters and numbers mark identified neurons

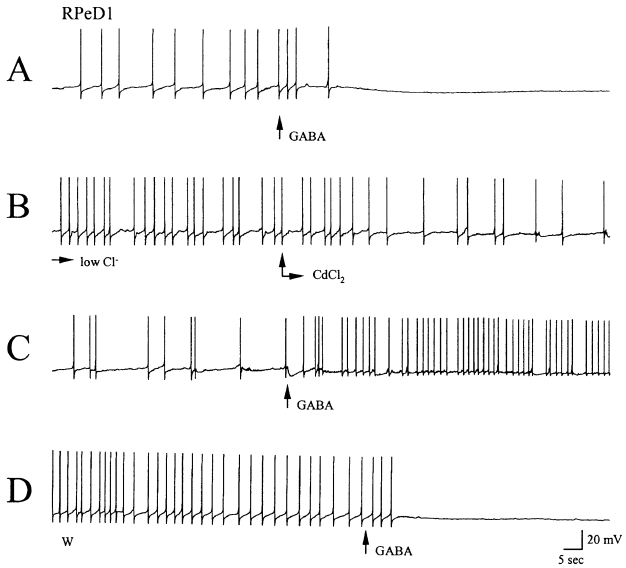

Fig. 2. Effect of GABA on the RPeD1 neuron in normal saline and in low chloride – cadmium containing solution. A – application of GABA (10^{-3} mol/l in the micro-pipette) to the neuron; B – activity of the neuron in low-Cl^- and 50 µmol/l cadmium solution; C – effect of application of GABA in low-Cl^-, cadmium containing solution. The evoked activity increase suggests the activation of other than Cl-channels. D – wash with normal saline (W) and repeated application of GABA. Here and in the following Figs the records are consecutive sections fragments from the same experiment

glia, and their reaction to GABA was tested. When a response was seen, the testing was repeated after 1–2 min pretreatment with $CdCl_2$ or the GABA antagonists bicuculline and picrotoxin. The isolated ganglion was placed in a small chamber allowing perfusion of the preparation and wash out of the drug after testing. For pretreatment with $CdCl_2$ and the toxins the whole ganglion was flooded with the solution, and GABA was applied in its presence. Application of GABA was performed with a micro-pipette, close to the neuron, in a volume of 100 µl. There was at least a 10 min interval between each successive GABA application. Resting and action potentials of the neurons were registered by a Gould–Brush chart recorder.

(b) Ion currents were measured by two-microelectrode voltage clamp technique on the cells RPeD1, RPaD1 and VD1 (Fig. 1), all sensitive to GABA. In the experiments a DAGAN 8500 voltage clamp amplifier was used with a custom written acquisition software to generate command steps and to store currents on an IBM compatible computer. GABA and other drugs were added directly to the surface of the examined cell from a plastic capillary with micro-perfusion for 2–3 secs, while at during the application of $CdCl_2$ and subsequent wash out the whole preparation was perfused. An interval of 5 min was included between drug applications to avoid desensitization of the receptors. With the exception of special measurements the holding potential (V_h) was –75 mV.

Statistical analysis

The data are given as arithmetic means with standard error of the mean. Statistical analyses were made by t-tests with $P < 0.05$ as significance criterion.

RESULTS

Effect of GABA on the potential generation of selected Lymnaea *neurons*

In current clamp experiments all the investigated neurons showed spontaneous activity with varying firing patterns. Depending on the neuron either pacemaker type, or clearly synaptically evoked, or mixed series of action potentials were seen. The resting potential (RP) varied between –35 and –55 mV, which did not change during the experiment, lasting for about two hours. In each experiment we normally recorded from two neurons simultaneously, and first tested for synaptic connections between them. Only neurons with no connections were used for subsequent tests. In a number of cases extracellular potentials from the right internal parietal nerve (i.p.n.) were recorded, to monitor the output activity of central neurons during drug treatment.

Each selected neuron was tested several times in different preparations, to establish if they responded to GABA. After preliminary trials with different concentrations we choose a 10^{-3} mol/l pipette concentration for the tests with GABA, with an estimated dilution factor of between 50 and 100 by the time it reached the cell mem-

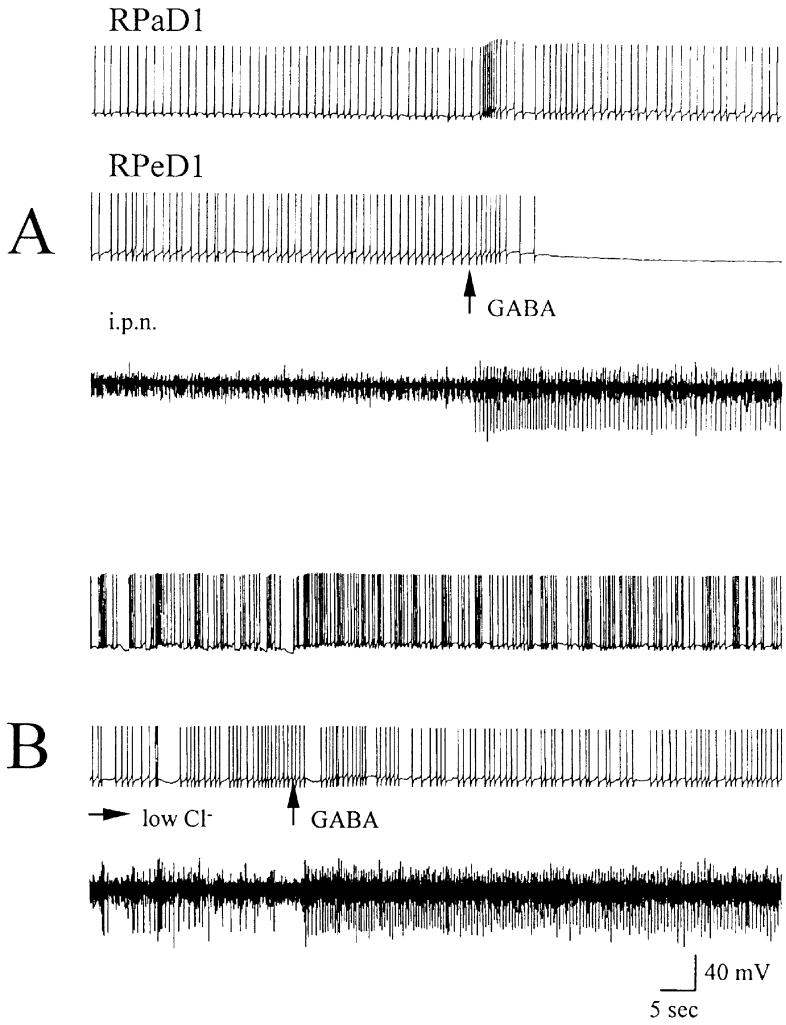

Fig. 3. Effect of GABA on the RPaD1 and RPeD1 neuron in normal saline and in low-Cl^- solution. A – In normal saline GABA application (10^{-3} mol/l in the micro-pipette) evoked depolarization with short excitation in the RPaD1 neuron and a hyperpolarization with long inhibition in the RPeD1 neuron; B – GABA application in low-Cl^- solution. Response of the RPaD1 neuron to GABA was partially reduced, while that of the RPeD1 neuron was totally prevented. On the i.p.n. a high amplitude, frequent activation appeared at GABA application both in normal and in low-Cl^- saline

brane. When there was an effect, it lasted for shorter or longer periods of time, up to 60–80 sec, and declined with or without washing. Nevertheless, each the preparation was washed out with physiological saline after each initial test with GABA, or, after subsequent tests, with the solution used for influencing the GABA effect ($CdCl_2$, low-Cl^- saline, GABA antagonists).

In response to GABA application, three main types of changes were observed in the resting potential and potential generation and these were characteristic to the neuron type tested:

(1) An immediate hyperpolarization, followed by inhibition of the ongoing spike activity (Fig. 2A and Fig. 3A; neuron RPeD1). The hyperpolarization reached an amplitude of 2–4 mV, and lasted, with a gradual reduction, for 1–2 min. After wash out, the original resting potential and activity whereas restored rapidly. This type of response was characteristic mainly to the neurons RPaD1, RPeD1, VV1, VV2 and three unidentified cells located in the vicinity of the neurons RPaD2, VD1 and VD2.

(2) Depolarization with an increase of the spike density of the neuron (Fig. 3A – RPaD1, Fig. 4A – RPaD2, and Fig. 5A – J neuron). This burst-type response was of a relatively short duration, lasting for 5–10 sec. The membrane depolarization was of 3–5 mV amplitude. A depolarizing response was characteristic to the neurons VD1, J and RPaD2, but occassionally also occurred in RPaD1.

(3) Often GABA application often evoked a biphasic response, starting with a short depolarization (3–5 mV) and an increase of the firing rate, followed within 5–10 sec by hyperpolarization and block of potential generation (Fig. 5A – RPeD1 neuron). In this effect inhibition was dominant, lasting for 1–2 min. No neuron was found among the 15 tested that would respond in each experiment exclusively by a biphasic response. However, some cell types, such as RPeD1, showed this type of response in nearly half of the cases (eight of eighteen). Occasionally the neurons RPaD1, RPaD2 and VD3 also responded to GABA in such a way.

Each type of response was consistent within the same experiment at repetition or after wash out. However, not all the neurons were sensitive to GABA, of the tested neurons VD2, LPaD2 and those of the F-cluster failed to react to GABA application.

Effect of Cd^{2+} on the GABA evoked neuronal response

In the presence of $CdCl_2$ the effect of GABA was modified, namely the response became weaker, was abolished and/or reversed (Fig. 5A and B, Fig. 2A and D; neuron RPeD1) Under the effect of Cd^{2+}-ions, the GABA evoked excitation was never reversed into inhibition, but the spiking rate of the cell could increase (Fig. 5A and B; J neuron). The effect of the Cd^{2+} was concentration dependent: at 10 µmol/l a weak or no influence was observed, 50 µmol/l caused a well expressed change in the GABA response without significantly modifying the control activity, while in 100 µmol/l or higher concentrations $CdCl_2$ depressed the potential generation. Therefore in the experiments we applied $CdCl_2$ in 10 and 50 µmol/l concentration.

It is generally accepted that the inhibitory effect of GABA is the result of Cl^- channel activation through $GABA_A$ receptors [34]. Therefore, we studied the influence of low-Cl^- solution on various GABA responses in both the absence and the presence of $CdCl_2$. As a rule, reduction of Cl^- in the saline did not modify the potential generating ability or the firing frequency of the cells studied.

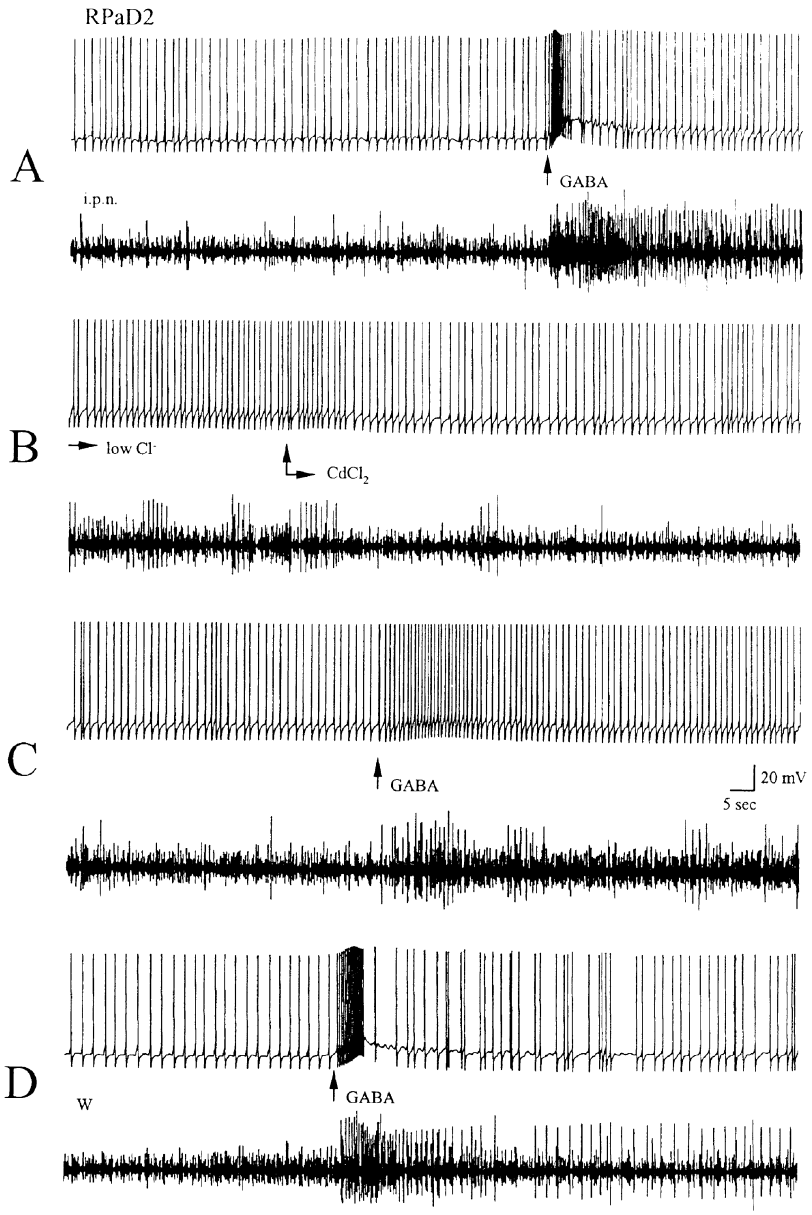

Fig. 4. Effect of GABA on the spike activity of RPaD2 neuron and of the i.p.n. nerve in normal saline and in low chloride – cadmium containing solution. A – GABA application (10^{-3} mol/l in the micropipette) evoked depolarization and a short excitation in the RPaD2 neuron, and an increase in the frequency of high large amplitude extracellular potentials on the i.p.n. B – spike activity of the neuron in low chloride – cadmium containing solution became less frequent. C – in low chloride – cadmium containing solution the effect of repeated GABA application was reduced as compared to A. Also the i.p.n. response was reduced. D – after wash out with normal saline (W), the effect of GABA effect returned to the control level

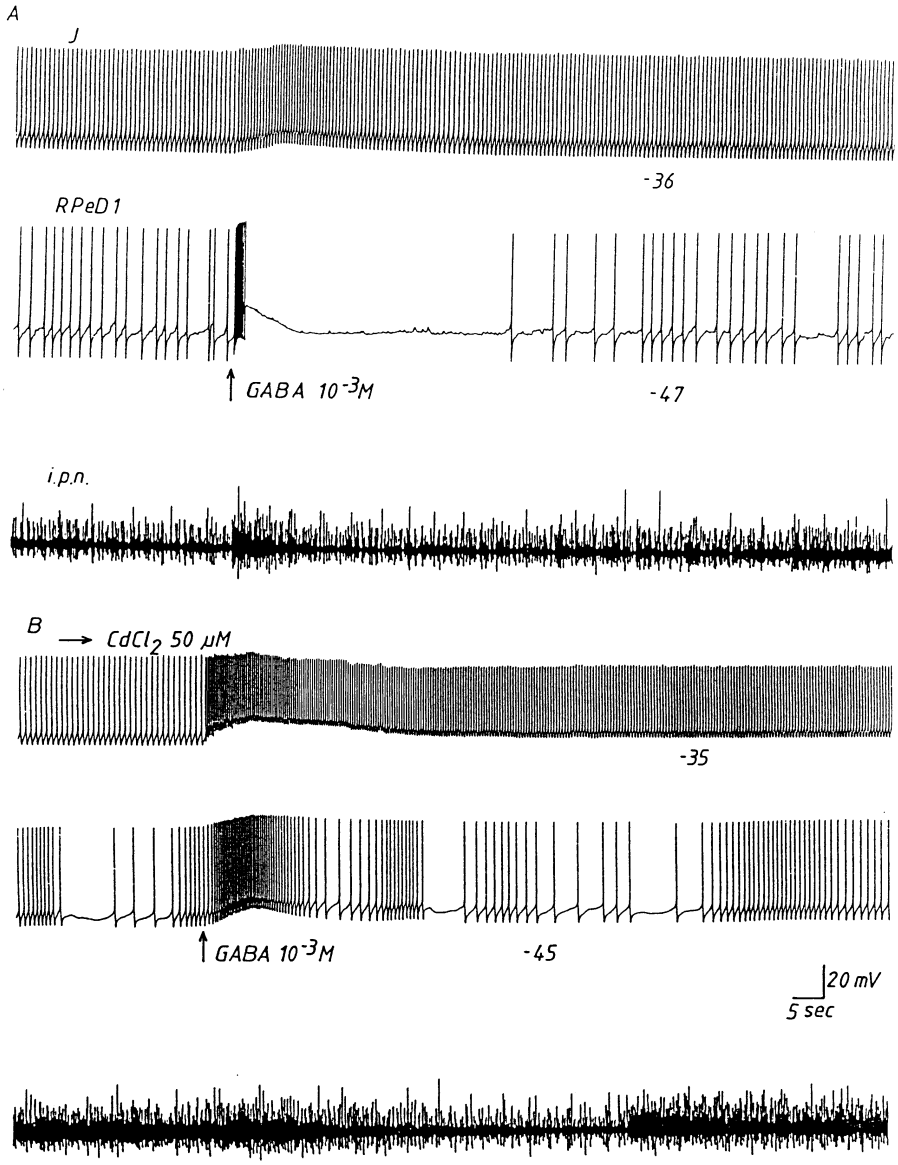

Fig. 5. Effect of GABA in normal (A) and in cadmium containing saline (B) on the J and RPeD1 neurons. In the presence of 50 µmol/l $CdCl_2$ the excitatory effect of GABA was augmented in both neurons, while the inhibition on RPeD1 neuron was abolished. The i.p.n. activity, increasing in the control at GABA application (A), was absent or reduced in the presence of cadmium (B)

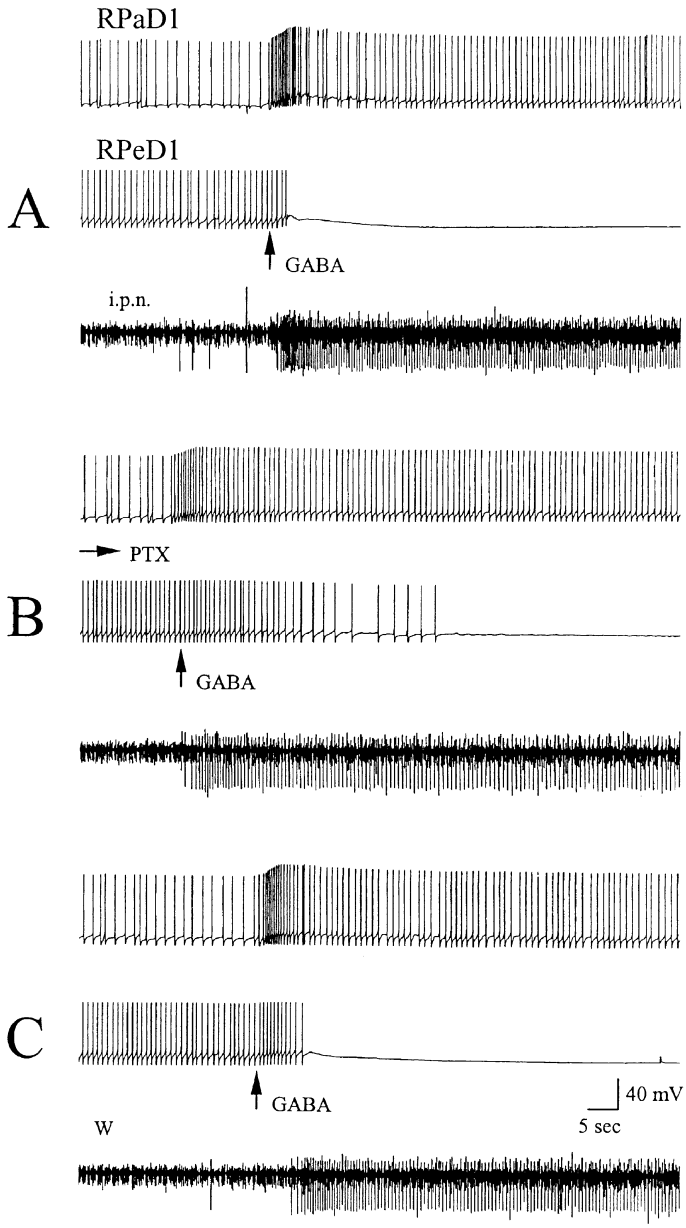

Fig. 6. Effect of picrotoxin (PTX) on the GABA evoked responses in the RPaD1 and RPeD1 neuron. A – GABA evoked short excitation in RPaD1, and long inhibition in RPeD1 neuron. B – in the presence of 10 µmol/l picrotoxin, the excitatory response to GABA was slightly reduced, however, the inhibitory response was blocked for prevented at least for 30 sec. C – after wash (W), the response to of GABA returned to the control level (A). In the activity of i.p.n. strong excitation appeared at GABA application both in normal saline and in the presence of picrotoxin

In low-chloride solution, both excitatory and inhibitory GABA responses were altered. GABA evoked depolarization was partially reduced in the cell RPaD1, while the long lasting hyperpolarization in neuron RPeD1 was entirely absent (Fig. 3A and B). In this latter neuron, when kept in low-Cl^- solution and treated with $CdCl_2$, not only was GABA induced inhibition prevented, but a slight excitation appeared (Fig. 2C). This can be the result of activation of an excitatory input from a neuron responding to GABA with depolarization, which is not effective when hyperpolarization is present in the target neuron. After wash out the inhibitory effect of GABA returned to the control level (Fig. 2D). In the neuron RPaD2 the short excitation evoked by GABA was reduced significantly under the influence of $CdCl_2$ in low-Cl^- saline (Fig. 4). The GABA effect returned to control after wash with SPS.

In vertebrates the chloride-channel blocker picrotoxin (PTX) and the $GABA_A$ receptor antagonist bicuculline were found to inhibit the effect of GABA. We tested both drugs for their potential influence on GABA evoked excitation and/or inhibition in *Lymnaea* neurons. In these experiments the RPaD1 neuron, responding with excitation, and the RPeD1 neuron, responding with inhibition, were studied. It was found, that PTX in 10 μmol/l concentration slightly depressed excitation and reduced, but failed to eliminate GABA induced inhibition (Fig. 3B). On the other hand, bicuculline had no effect on the GABA evoked responses.

In some cases, together with recording from identified neurons, also the extracellular activity of the right internal parietal nerve (Fig. 1., IPan) also was recorded. This nerve carries information from the right parietal ganglion to the osphradium and its activity is a good indicator of excitatory and inhibitory processes taking place in the neural network of the ganglia [29]. When GABA was applied to the isolated ganglion an increase of activity occurred on this nerve, outlasting the effect observed on the neurons tested (Figs 3., 4. and 5). The activity increase of the nerve was characteristic, and occurred independently from the depolarization or hyperpolarization that was induced by GABA on the studied neurons. Therefore, it clearly did not originate from the cells tested, but represented the activity of other neurons, responding to GABA with excitation.

Effect of Cd^{2+} on the GABA induced Cl^--current

In accordance with earlier findings [25, 26], at a –75 mV holding potential GABA application evoked an inward Cl^--current, characterized by a fast rising phase and a much slower decay on neurons RPaD1 and RPeD1 (Fig. 7). The amplitude of the current varied between 10–15 nA in different experiments and neurons, but was rather consistent in the same cell at repeated GABA applications of a given concentration. The response was concentration dependent in the 25 and 500 μmol/l range. In this range the amplitude of the current could increase by 500 per cent (Fig. 7A). For our detailed studies we choose the current evoked by 25 μmol/l GABA.

Applying 25 μmol/l GABA the holding potential dependence of the response was studied. With changing the V_h towards positive direction, the amplitude of the

response decreased and the reversal potential was reached around –10 mV (Fig. 7B), corresponding closely to E_{Cl}. For testing the specificity of the GABA response as compared to vertebrate systems, we studied the effect of PTX and bicuculline in our preparation. Application of 0.1 mmol/l PTX yielded a 65 per cent blocking of GABA activated ion current, however, bicuculline was ineffective in this concentration.

The effect of $CdCl_2$ on the GABA evoked inward current was studied in 1, 5, 10 and 50 µmol/l concentrations. The ganglion was perfused with cadmium containing

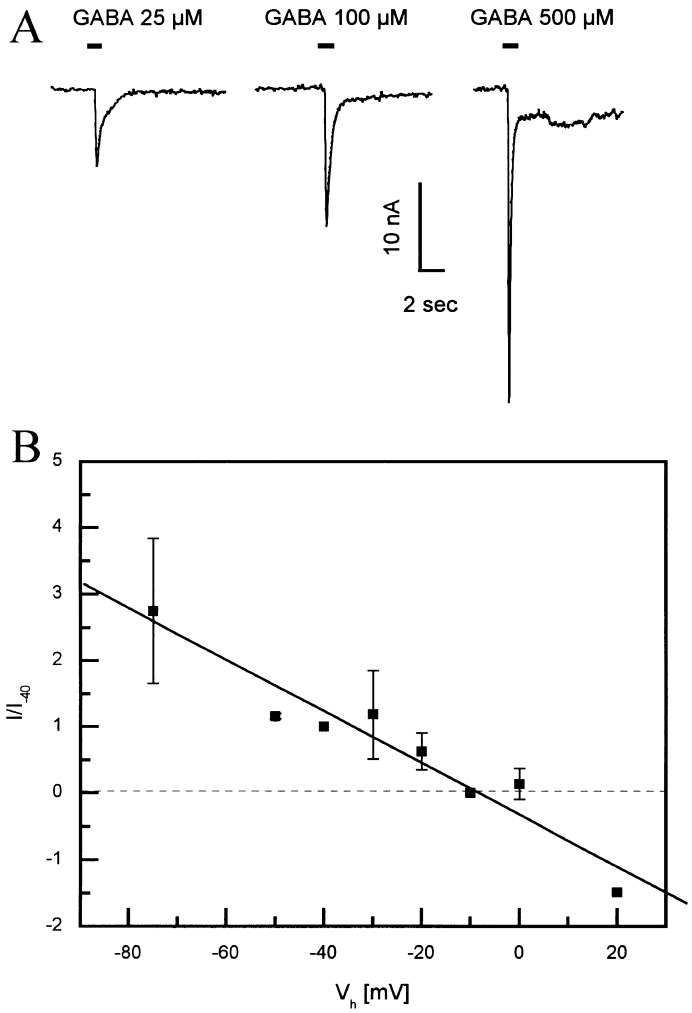

Fig. 7. GABA evoked currents and I/V characteristics. A – inward Cl^--currents evoked by 25, 100 and 500 µmol/l GABA concentrations at –75 mV holding potential. Current amplitudes are 8.6, 15.8 and 40.4 nA, respectively. B – current – voltage characteristics of normalized GABA response. Currents were normalized to peak value of the inward current induced at –40 Vh

saline for 30 min, and the response to GABA was tested with at 5 min intervals. There was a clear concentration and time dependency in the response: by the end of the treatment 1 µmol/l $CdCl_2$ caused a small (10 per cent) increase in the current, while higher concentrations resulted in depression up to the point of totally preventing the GABA effect (Fig. 8). The inhibitory effect of Cd^{2+} was not linear with time. The enhancement caused by 1 µmol/l $CdCl_2$ showed some variation in time, the effect of 5 µmol/l $CdCl_2$ was more or less linearly time dependent throughout the

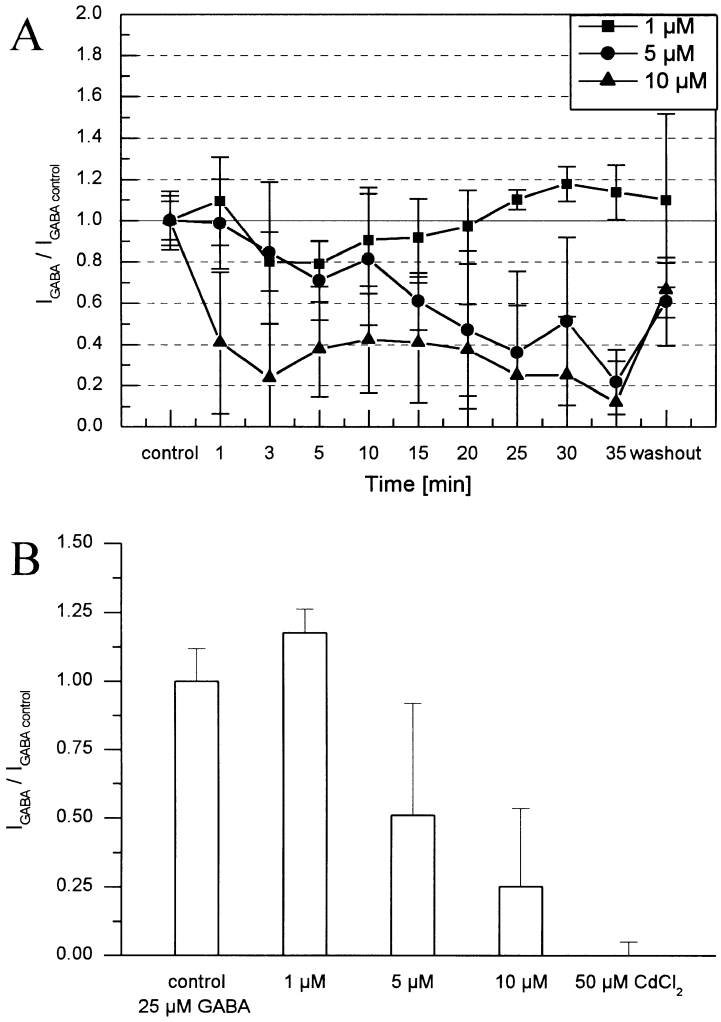

Fig. 8. Modulatory effect of $CdCl_2$ on the GABA evoked Cl^--current. A – effect of 1–5–10 µmol/l $CdCl_2$ on the GABA evoked current, for 35 min. B – comparison of the effects of 1–5–10–50 µmol/1 $CdCl_2$ in the 30th minute of the application

treatment, while the inhibitory effect of 10 µmol/l $CdCl_2$ reached its maximum (60–80 per cent) within three min and remained in this range by the end of the treatment (Fig. 8A). After 30 min wash out the control returned only partially following 5 and 10 µmol/l $CdCl_2$ treatment.

Under the effect of Cd^{2+} not only did the amplitude of the GABA evoked current changed, but also the activation and inactivation time underwent to alterations. In the presence of 10 µmol/l $CdCl_2$, the time to peak was nearly doubled, while the half inactivation time increased by 30 per cent, within 20 min (Fig. 9).

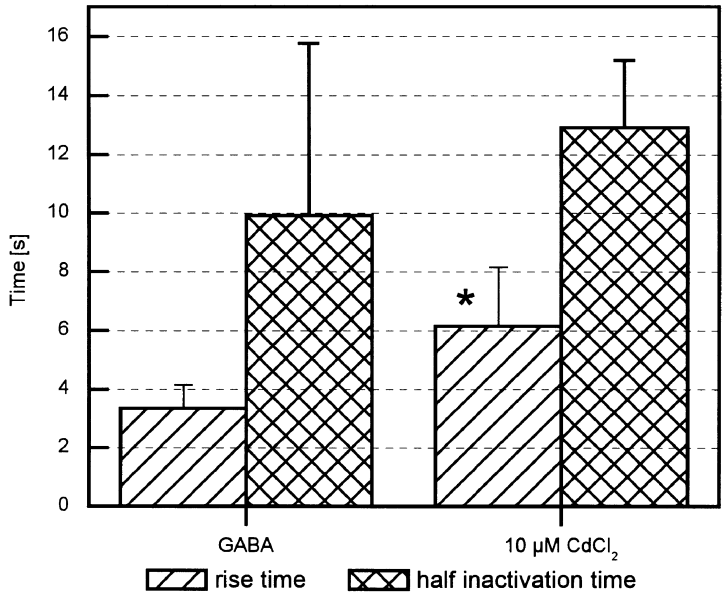

Fig. 9. Effect of 20 minutes of acute application of $CdCl_2$ onto the rising and half-inactivation times of the GABA induced current. Time-to-peak in control was 3.3±0.81 sec and after Cd^{2+} treatment 6.15±2.01 sec. Half inactivation time was 9.9±5.85 sec in control and 12.92±2.26 sec after treatment. *significancy at $P < 0.05$

It is known, that heavy metal ions, and among them under some conditions Cd^{2+}, are able to evoke inward or outward currents ion neuronal membranes (see rev. [17]). In combined experiments, the metal induced current may summate with that of the transmitter evoked one. Therefore we studied the effect of $CdCl_2$ at a –75 mV holding potential in the concentration that was found to affect the GABA current. We found that application of 50 µmol/l $CdCl_2$ induced a slow inward current of 1–2 nA (Fig. 10). This could be predicted to increase the GABA evoked current through a process of summation. However, the inward GABA current was not augmented, but rather it was reduced in the presence of Cd^{2+}-ions, proving that Cd^{2+} has an inhibitory influence on the membrane effect of GABA.

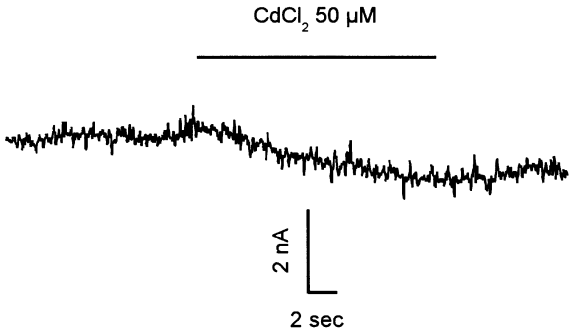

Fig. 10. Effect of $CdCl_2$ on the cell membrane at –75 mV holding potential. The cell was under continuous SPS perfusion and $CdCl_2$ was added by microperfusion (solid line) onto the cell surface. An inward current of 1–2 nA was induced during Cd^{2+} application

DISCUSSION

GABA is one of the major neurotransmitters in the animal kingdom and is regarded as a mainlyn inhibitory substance in the central nervous system of adult vertebrates. However, it can also be excitatory in the human embryo [12]. In lower animals, especially in molluscs, inhibitory and excitatory actions of GABA may exist in parallel in various neurons [18]. In *Aplysia* nerve cells, according to Yarowsky and Carpenter [37], GABA may evoke five types of responses with the involvement of either chloride, potassium or sodium ions. In mammals, three types of GABA receptors have been distinguished pharmacologically. The most widely studied is the $GABA_A$ type, gating ionotropic chloride channels, while $GABA_B$ type acts via G-proteins on K^+- and Ca^{2+}-channels, and the $GABA_C$ receptor is involved in metabotropic activation of Cl^--channels, resulting all in inhibition. Excitation was ascribed to activation of Na^+-channels [3, 37]. By using molecular biological techniques the $GABA_A$ receptor of *Lymnaea* has been cloned, and a 35–47 per cent identity with vertebrate ligand gated Cl^--channel receptor subunits was found [7]. Together with pharmacological characteristics [26, 34] it indicates similarities, but also some differences between vertebrate and invertebrate GABA receptors.

In our current clamp experiments at least three types of effects were recorded at GABA application, in two of them hyperpolarization, while in one depolarization dominated. A fourth type of GABA response, long lasting excitation, occurred under Cd^{2+} treatment in a neuron that was inhibited by GABA in physiological saline (Fig. 2). The inhibitory effect can be ascribed to Cl^--channel activation by $GABA_A$ receptors, while the short and well expressed excitatory effect to activation of a Na^+-current. The biphasic response (excitation/inhibition) can be the result of concomitant activation of Na^+- and Cl^--channels, when Na-activation is of a shorter duration. However, for this case another possibility should also be considered. In our experimental circumstances not only the selected neuron was reached by GABA, but also

neighboring cells, which, if depolarized, may evoke excitation synaptically in the neuron we were recording from, before inhibition became dominant under the effect of GABA. The activation of a number of unidentified neurons is demonstrated by the extracellular potentials recorded from the internal parietal nerve, which may provide inputs also to a number of neurons being members of the same or different neuronal networks [29].

Similarly to other studies on *Lymnaea* [25, 31], in our present voltage clamp experiments at a –75 mV holding potential we could record only an inward current in response to GABA from the selected neurons; this reversed at around –10 mV HP. This indicated that it can be neither a Na^+- nor a Ca^{2+}-current, but perhaps connected to Cl^--channel activation. This is supported by the fact that in current clamp experiments at –40 – –45 mV resting potential the inhibitory GABA effect was reduced or eliminated in low-Cl^- solution, which returned to control levels in SPS. Of the GABA antagonists used both in the current clamp and in voltage clamp experiments only picrotoxin was effective in low concentration, but bicuculline did not prevent GABA effect. Similar results were reported also in other invertebrate neurons [34], referring to differences of vertebrate and invertebrate $GABA_A$ receptors.

The modulation of GABA effect by various substances have been shown previously, among others by a number of heavy metals in rat dorsal ganglion neurons [19, 21], and also in nerve cells of *Lymnaea* [25]. GABAergic transmission and GABA evoked currents were modulated also by various signal molecules, *e.g.* by serotonin in pyramidal [24], and by enkephalins in molluscan neurons [31].

Among toxic metals, which may interact with neural regulation cadmium deserves special attention, due to a permanent occupational and environmental concern about its effects. Cadmium may result in various health disorders, being most striking the so called itai-itai disease in Japan [22], caused by cadmium rich mine effluent used for rice field irrigation. A range of neurological disorders as a consequence of cadmium exposure has also been reported [6]. Cadmium is used commonly in neurophysiological experiments as a specific Ca^{2+}-channel blocker, however, it proved to block also K^+- and Cl^--channels [19, 27], both in vertebrate and molluscan neurons.

Our present recent results indicate Cd^{2+} in low concentration has a depressive effect on GABA induced potential generation and an inhibitory effection on Cl^--currents in *Lymnaea* neurons. In this respect it is in agreement with findings reported earlier in turtle retina [16], in rat dorsal root ganglia [19] and also with the results of Rubakhin et al. [26] obtained on isolated *Lymnaea* neurons. However, we found the a depression of Cl^--current in the snail neurons to be a hundred times lower at a 10 µmol/l $CdCl_2$ concentration and observed augmentation at a 1 µmol/l concentration.

The inhibition caused by $CdCl_2$ on the GABA evoked potential generation, studied in current clamp experiments, was restricted to the hyperpolarizing response, but the excitatory was only slightly influenced, or was not modified at all. This can be explained by the effect of Cd^{2+} on the Cl^--channels responsible for inhibition, while the channels responsible for the excitatory effect of GABA were not influenced.

There exist several possible explanations for how Cd^{2+} can modulate the GABA induced ion-currents, inasmuch as it can have a variety of targets. The main possibilities are: (a) by the prevention channel opening by binding to the GABA-receptor complex, (b) by the obstruction of the external or internal orifice of the ion channel, (c) by affecting internal metabolic processes and the second messenger system.

The binding to the GABA-receptor complex and resulting either modification of the gating mechanism or the closing of the channel for the movement of chloride ions are both potential mechanisms for blocking the Cl^--current. Modification of the receptor by binding to one of the subunits seems to be a more plausible explanation, because it can led not only to suppression, but also may result in augmentation, as it was suggested by Ma and Narahashi [19] for the enhancement of GABA induced Cl^--currents by lanthanum. Nevertheless, the possibility exists, that enhancement by low cadmium is caused by mechanism other than suppression of Cl^--channels, which may take place in a similar way to how tetrodotoxin blocks Na^+-channels [13]. Occlusion of voltage gated chloride channels by cadmium was shown in cultured choroid plexus epithelial cells [15] and in bipolar cells of rat retina [8].

The possible interaction with intracellular processes and the second messenger system for the explanation of the inhibitory effect of Cd^{2+} may be linked to the inward current caused by cadmium itself on the membrane. It was shown in cultured renal epitheloid cells that cadmium induced inward current enhances potassium conductance by increasing intracellular free Ca^{2+} [14]. Extracellular application of divalent cations on *Xenopus* oocytes elicited oscillatory Cl^--currents [20] through an intracellular messenger pathway involving inositol phosphates. This Cl^--channel activation was caused also by mobilization of Ca^{2+} from intracellular stores. Furthermore, there are experiments showing that the GABA activated Cl^--current is depressed by increased intracellular Ca^{2+}-concentration [1, 16]. Thus, it can be suggested that extracellularly applied Cd^{2+} inhibits GABA evoked Cl^--current in an indirect way, namely, by increasing intracellular Ca^{2+}. Further studies are needed to verify this possibility.

GABA mediated inhibitory processes are considered to be of fundamental importance in neural regulation in of both vertebrate and invertebrate nervous systems. Recently, it has been shown in rat neocortex that GABAergic transmission from interneurons to the pyramidal cells may evoke a variety of IPSP patterns at the postsynaptic membrane [9] allowing very effective regulation of the activity of the follower cells through specific spatiotemporal patterns. This way the GABAergic system plays a central role in finetuned, distinctive regulatory mechanisms. Even a slight interference of external substances, such as like Cd^{2+}, with one or the other type of GABAergic response may cause changes in the regulatory mechanisms. The clarification of these possible influences can contribute to the understanding of the neuropathological effects caused by toxic substances.

ACKNOWLEDGMENT

Authors express many thanks to to Eugénia B.-Hollósy for her skillful technical assistance, and to György Kemenes for reading the manuscript. This work was supported by an the OTKA grant (T020944) to J. Salánki.

REFERENCES

1. Akopian, A., Gabriel, R., Witkovsky, P. (1998) Calcium released from intracellular stores inhibits $GABA_A$-mediated currents in ganglion cells of the turtle retina. *J. Neurophysiol. 80,* 1105–1115.
2. Arakawa, O., Nakahiro, M, Narahashi, T. (1991) Mercury modulation of GABA-activated chloride channels and non-specific cation channels in rat dorsal root ganglion neurons. *Brain Res. 551,* 58–63.
3. Bokisch, A. J., Walker, R. J. (1986) The ionic mechanism associated with the action of putative transmitters on identified neurons of the snail, *Helix aspersa. Comp. Biochem. Physiol. 84C,* 231–241.
4. Büsselberg, D., Michael, D., Platt, B. (1994) Pb^{2+} reduces voltage- and N-methyl-D-aspartate (NMDA)-activated calcium channel currents. *Cell. Mol. Neurobiol. 14,* 711–722.
5. Carpenter, D. O. (1994) The public health significance of metal neurotoxicity. *Cell. Mol. Neurobiol. 14,* 591–597.
6. Chang, L. W. (ed.) *Toxicology of Metals.* CRC Press, Inc., Lewis Publisheres, New York, USA, 1996.
7. Darlison, M. G., Hutton, M. L., Harvey, R. J. (1993) Molluscan ligand-gated ion-channel receptors. In: Pichon, Y. (ed.) *Comparative Molecular Neurobiology.* Birkhauser Verlag, Basel, Switzerland, pp. 48–64.
8. Enz, R., Ross, B. J., Cutting, G. R. (1999) Expression of the voltage-gated chloride channel ClC-2 in rod bipolar cells of the rat retina. *J. Neurosci. 19,* 9841–9847.
9. Gupta, A., Wang, Y., Macram, H. (2000) Organizing principles for a diversity of GABAergic interneurons and synapses in the neocortex. *Science 287,* 273–278.
10. Győri, J., Kiss, T., Shcherbatko, A. D., Belan, P. V., Tepikin, A. V., Osipenko, O. N., Salánki, J. (1991) Effect of Ag^+ on membrane permeability of perfused *Helix pomatia* neurons. *J. Physiol. (London) 442,* 1–13.
11. Győri, J., Fejtl, M., Carpenter, D. O., Salánki, J. (1994) Effect of $HgCl_2$ on acetylcholine, carbachol, and glutamate currents of *Aplysia* neurons. *Cell. Mol. Neurobiol. 14,* 653–664.
12. Herlenius, E., Lagercrantz, H. (2001) Neurotransmitter and neuromodulators during early human development. *Early Hum. Dev. 65,* 21–37.
13. Hille, B. (1975) The receptor for tetrodotoxin and saxitoxin. A structural hypothesis. *Biophys. J. 15,* 615–619.
14. Jungwirth A., Paulmichl, M., Lang F. (1990) Cadmium enhances potassium conductance in cultured renal epitheloid (MDCK) cells. *Kidney International 37,* 1477–1486.
15. Kajita, H., Omori, K., Matsuda, H. (2000) The chloride channel ClC-2 contributes to the inwardly rectifying Cl^- conductance in cultured porcine choroid plexus epithelial cells. *J. Physiol (London) 523,* 313–324.
16. Kaneko, A., Tachibana, M. (1986) Blocking effect of cobalt and related ions on the gamma-aminobutyric acid-induced current in turtle retinal cones. *J. Physiol. (London) 373,* 463–479.
17. Kiss, T., Osipenko, O. N. (1994) Toxic effects of heavy metals on ionic channels. *Pharmacol. Rev. 46,* 245–267.
18. Leake, L., Walker, R. J. (1980) *Invertebrate Neuropharmacology,* Blackie, Glasgow and London, U.K.
19. Ma, J. Y., Narahashi, T. (1993) Differential modulation of $GABA_A$ receptor-channel complex by polyvalent cations in rat dorsal root ganglion neurons. *Brain Res. 607,* 222–232.
20. Miledi, R., Parker, I., Woodward, R. M. (1989) Membrane currents elicited by divalent cations in *Xenopus* oocytes. *J. Physiol. (London) 417,* 173–195.

21. Narahashi T., Ma, J. Y., Arakawa, O., Reuveny, E., Nakahiro, M. (1994) GABA receptor-channel complex as a target site of mercury, copper, zink, and lanthanides. *Cell. Mol. Neurobiol. 14,* 599–621.
22. Nogawa, K., Kido, T. (1966) Itai-itai disease and health effects of cadmium. In: Chang, L. W. (ed.) *Toxicology of Metals.* Lewis Publishers, New York, USA, pp. 353–369.
23. Oortgiesen, M., van Kleef, R. G. D. M., Bajnath, R. B., Vijverberg, H. P. M. (1990) Novel type of ion channel activated by Pb^{2+}, Cd^{2+} and Al^{3+} in cultured mouse neuroblastoma cells. *J. Membr. Biol. 113,* 261–268.
24. Ropert, N., Guy, N. (1991) Serotonin facilitates GABAergic transmission in the CA1 region of rat hippocampus *in vitro*. *J. Physiol. (London) 441,* 121–136.
25. Rubakhin, S. S., Győri, J., Carpenter, D. O., Salánki, J. (1995) $HgCl_2$ potentiates GABA activated currents in *Lymnaea stagnalis* neurones. *Acta Biol. Hung. 46,* 431–444.
26. Rubakhin, S. S., Szűcs, A., S.-Rózsa, K. (1996) Characterization of the GABA response on identified dialysed *Lymnaea* neurons. *Gen. Pharmac. 27,* 731–739.
27. Salánki, J. (1988) Invertebrates in neuroscience. In: Salánki, J., S.-Rózsa, K. (eds) *Neurobiology of Invertebrates. Transmitters, Modulators and Receptors.* Akadémiai Kiadó, Budapest, Hungary, pp. 1–10.
28. Salánki, J., Győri, J., Carpenter, D. O. (1994) Action of lead on glutamate activated chloride currents in *Helix pomatia* L. neurons. *Cell. Mol. Neurobiol. 14,* 755–768.
29. S.-Rózsa, K. (2000) Modulation of firing pattern and oscillation in nerve cells of *Lymnaea* during network reconstruction. *Acta Biol. Hung. 51,* 211–230.
30. S.-Rózsa, K., Salánki, J. (1985) Effects of heavy metals on the chemosensitivity of neuronal somata of *Lymnaea stagnalis* L. In: Salánki, J. (ed.) *Heavy Metals in Water Organisms.* Akadémiai Kiadó, Budapest, Hungary, pp. 387–400.
31. S.-Rózsa, K., Rubakhin, S. S., Szűcs, A., Stefano, G. B. (1996) Met-enkephalin and morphiceptin modulate a GABA-induced inward current in the CNS of *Lymnaea stagnalis* L. *Gen. Pharmac. 27,* 1337–1345.
32. S.-Rózsa, K., Salánki, J. (1987) Excitable membranes – object for evaluating the effect of heavy metal pollution. *Acta Biol. Hung. 38,* 31–45.
33. S.-Rózsa, K., Salánki, J., Présing, M. (1988) Use of *Lymnaea stagnalis* in monitoring heavy metal pollution. In: Yasuno, M., Whitton, B. A. (eds) *Biological Monitoring of Environmental Pollution.* Tokai University Press, Tokyo, Japan, pp. 247–255.
34. Walker, R. J., Brooks, H. L., Holden-Dye, L. (1996) Evolution and overview of classical transmitter molecules and their receptors. *Parasitology 113,* S3–S33.
35. Weinreich, D., Wonderlin, W. F. (1987) Copper activates a unique inward current in molluscan neurones. *J. Physiol. (London) 394,* 429–443.
36. Winlow, W., Benjamin, P. R. (1976) Neuronal mapping of the brain of the pond snail, *Lymnaea stagnalis* L. In: Salánki, J. (ed.) *Neurobiology of Invetebrates. Gastropoda Brain.* Akadémiai Kiadó, Budapest, Hungary, pp. 41–59.
37. Yarowsky, P. J., Carpenter, D. O. (1978) Receptors for gamma-aminobutyric acid (GABA) on *Aplysia* neurons. *Brain Res. 144,* 75–94.