

Dopamine and Serotonin Modulate Human GABA ρ 1 Receptors Expressed in *Xenopus laevis* Oocytes

Lenin D. Ochoa-de la Paz,[†] Argel Estrada-Mondragón,^{†,‡} Agenor Limón,[§] Ricardo Miledi,[†] and Atáulfo Martínez-Torres^{*,†}

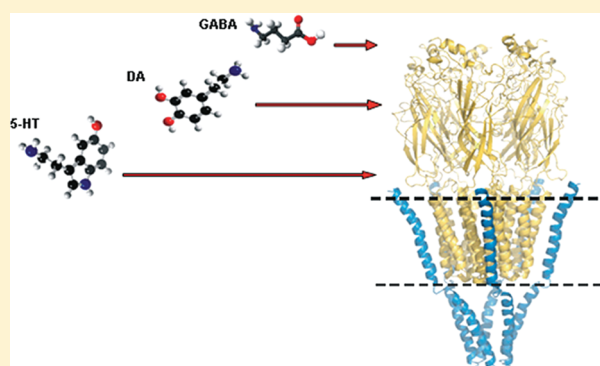
[†]Departamento de Neurobiología Celular y Molecular, Laboratorio de Neurobiología Molecular y Celular I-II, Instituto de Neurobiología, Campus UNAM Juriquilla, Querétaro, Qro. México CP 76230

[‡]Institut de Biologie Structurale Jean-Pierre Ebel, CEA-CNRS-UJF, 41 rue Jules Horowitz, F-38027 Grenoble, France

[§]Neurobiology and Behavior, University of California, Irvine, 2205 McGaugh Hall, Irvine California 92697, United States

ABSTRACT: GABA ρ 1 receptors are highly expressed in bipolar neurons of the retina and to a lesser extent in several areas of the central nervous system (CNS), and dopamine and serotonin are also involved in the modulation of retinal neural transmission. Whether these biogenic amines have a direct effect on ionotropic GABA receptors was not known. Here, we report that GABA ρ 1 receptors, expressed in *X. laevis* oocytes, were negatively modulated by dopamine and serotonin and less so by octopamine and tyramine. Interestingly, these molecules did not have effects on GABA $_A$ receptors. 5-Carboxamido-tryptamine and apomorphine did not exert evident effects on any of the receptors. Schild plot analyses of the inhibitory actions of dopamine and serotonin on currents elicited by GABA showed slopes of 2.7 ± 0.3 and 6.1 ± 1.8 , respectively, indicating a noncompetitive mechanism of inhibition. The inhibition of GABA ρ 1 currents was independent of the membrane potential and was insensitive to picrotoxin, a GABA receptor channel blocker and to the GABA ρ -specific antagonist (1,2,5,6-tetrahydropyridine-4-yl)methyl phosphinic acid (TPMPA). Dopamine and serotonin changed the sensitivity of GABA ρ 1 receptors to the inhibitory actions of Zn $^{2+}$. In contrast, La $^{3+}$ potentiated the amplitude of the GABA currents generated during negative modulation by dopamine (EC_{50} 146 μ M) and serotonin (EC_{50} 196 μ M). The functional role of the direct modulation of GABA ρ receptors by dopamine and serotonin remains to be elucidated; however, it may represent an important modulatory pathway in the retina, where GABA ρ receptors are highly expressed and where these biogenic amines are abundant.

KEYWORDS: GABA ρ 1, GABA $_A$, dopamine, receptor modulation, 5-HT, *Xenopus* oocyte



γ -Aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the adult mammalian brain and retina. The ionotropic receptors for GABA are ligand-gated chloride channels that are targets for a variety of clinically prescribed therapeutic compounds.¹ Among the different isoforms of GABA receptors, those made up by ρ 1–3 subunits (GABA ρ) display a pharmacological profile that is different from the more ubiquitous GABA $_A$ receptors composed of α , β , and γ subunits. GABA ρ receptors are blocked by picrotoxin (Ptx) and by (1,2,5,6-tetrahydropyridine-4-yl)methylphosphinic acid (TPMPA), and they are insensitive to bicuculline, barbiturates, and benzodiazepines.^{2–7}

GABA ρ subunits have been found in several regions of the central nervous system, including cerebellar Purkinje neurons^{8,9} and the amygdala.¹⁰ In Purkinje neurons, GABA ρ mediate a component of phasic inhibitory transmission,¹¹ whereas in the amygdala, pharmacological evidence suggests that GABA ρ -mediated activity participates in the modulation of fear and anxiety.¹⁰ In the retina, GABA ρ receptors are highly expressed

at the axon terminal of bipolar neurons where they are involved in regulating visual signaling.^{12,13}

Several studies have shown indirect modulation of GABA ρ by dopamine (DA) in horizontal cells of the catfish and in bipolar cells of the tiger salamander.^{14,15} Moreover, it has been suggested that G protein-coupled receptors, such as those for glutamate or serotonin (5-HT), indirectly reduce GABA ρ -mediated responses in bipolar cells.¹⁶ Because cross-talk between dopamine and serotonin G protein-coupled receptors has been reported,^{4,16,17} and because GABA ρ subunits are modulated by several molecules besides GABA,^{19–22} it is possible that DA or 5-HT directly interacts with GABA ρ receptors. However, in spite of their pharmacological importance, such interactions have not been explored.

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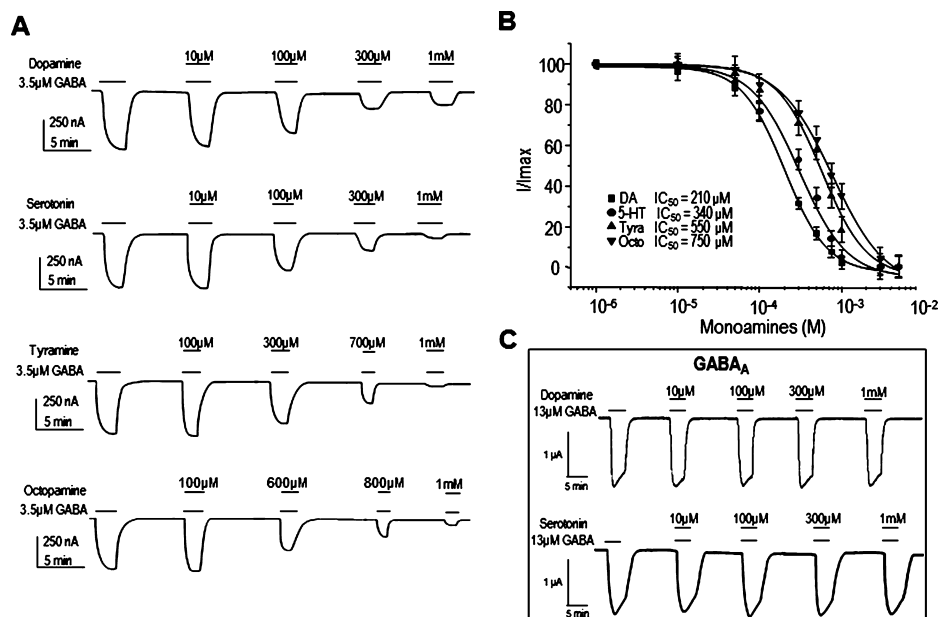


Figure 1. Effects of the biogenic amines on GABA-currents in oocytes expressing GABA ρ 1 receptors. (A) Effect of biogenic amines at several concentrations upon currents activated by 3.5 μ M GABA. (B) Monoamine concentration–response relationships showing the IC₅₀ for each one. Data were normalized to the maximal GABA response (3.5 μ M) of each oocyte (C) DA and 5-HT did not modulate GABA_A receptors. DA (■), 5-HT (●), Tyra (▲), and Octo (▼). Data points are the means \pm SEM obtained from at least 9 oocytes ($n = 9$) from 5 different frogs ($N = 5$).

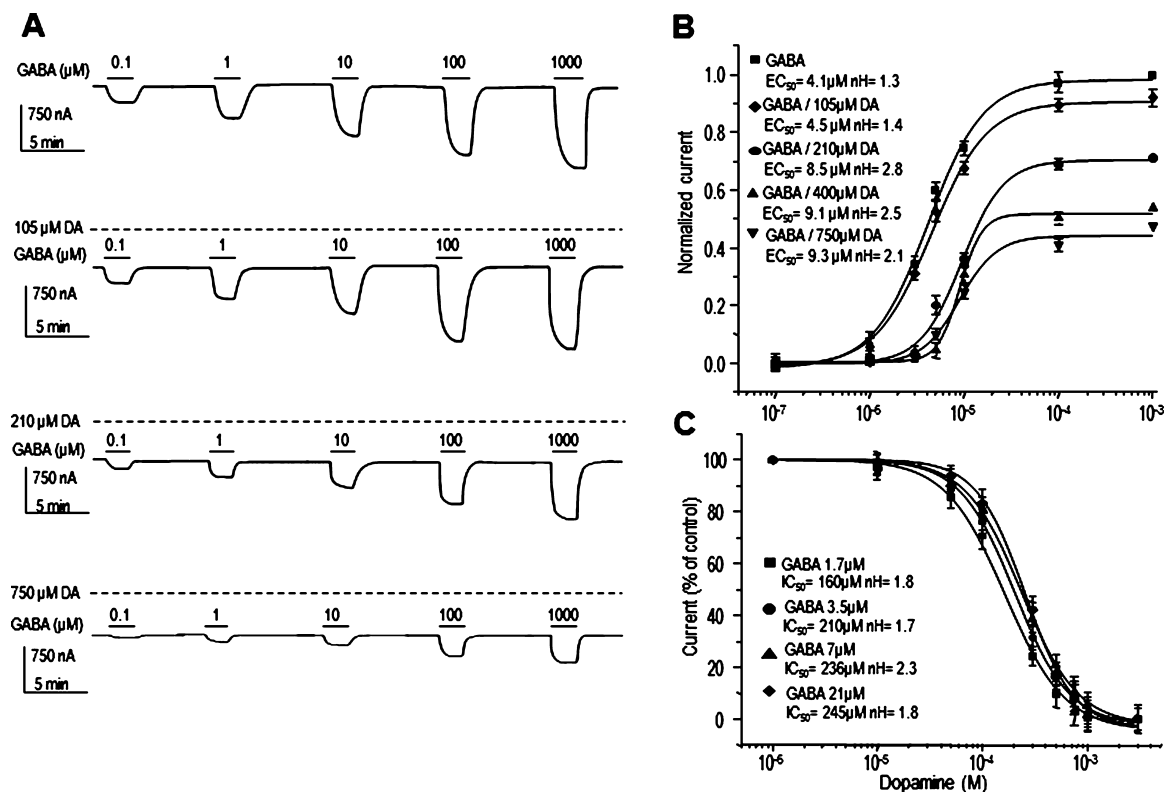


Figure 2. Competition assays. (A) Sample currents and (B) concentration–response relationships of GABA-current modulation by DA (105, 210, 400, and 750 μ M) in oocytes expressing GABA ρ 1. DA concentration–response curves of currents elicited by 1.7, 3.5, 7, and 21 μ M GABA. Notice that DA did not shift the GABA dose–response curve ($P > 0.05$). Data points are the means \pm SEM obtained from at least 9–11 oocytes from 5 frogs.

In previous studies, we have demonstrated a negative serotonergic modulation of nicotinic acetylcholine receptors in isolated muscle fibers and in cloned receptors.^{23,24} Here, we extend these observations to cloned GABA receptors, in which

we assessed the effect of biogenic amines. Our results suggest a direct inhibition of GABA ρ 1 by DA, 5-HT, tyramine, and octopamine. In contrast, this modulation was not observed for GABA_A receptors composed of α 1 β 2 γ 2 subunits. Thus, we

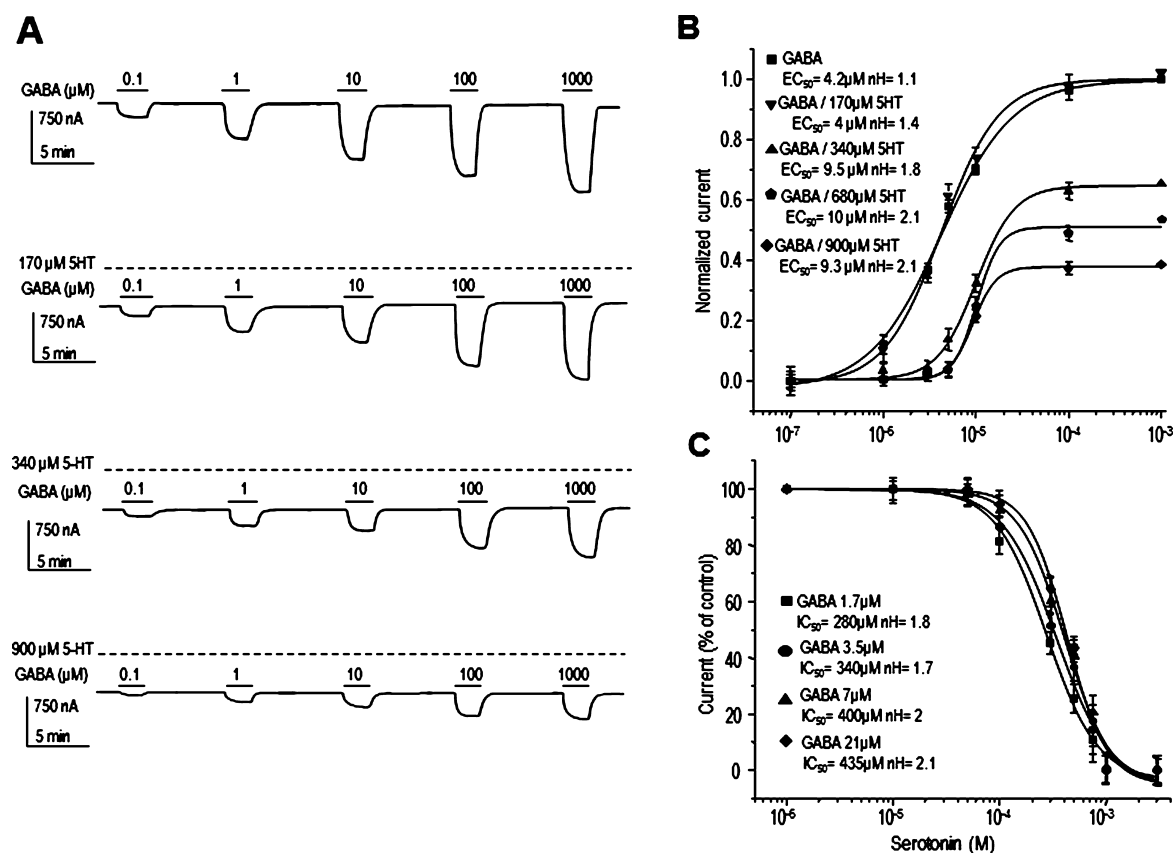


Figure 3. Competition assays. (A) Sample currents and (B) concentration–response relation of GABA-current modulation by 5-HT (140, 340, 680, and 900 μM) in oocytes expressing GABA ρ 1 (C) 5-HT concentration–response curves of currents elicited by 1.7, 3.5, 7, and 21 μM GABA. Notice that 5-HT did not affect the GABA dose–responses curve ($P > 0.05$). Data points are the means \pm SEM obtained from at least 5 oocytes ($n = 4–7$) from 5 frogs ($N = 5$).

found yet another peculiar characteristic of the GABA ρ subunits that sets them apart from the classic GABA $_A$ receptors and that may help in the design of new subunit-specific antagonists.

RESULTS AND DISCUSSION

Xenopus oocytes expressing GABA ρ 1 receptors generated typical nondesensitizing ionic currents of $1.4 \pm 0.5 \mu\text{A}$ ($n = 19$) upon perfusion of 1 mM GABA. The EC₅₀ for GABA was $3.5 \pm 0.4 \mu\text{M}$ ($n = 19$). The same oocytes did not generate detectable responses during perfusion of 300 μM DA or 5-HT. Similarly, perfusion of tyramine (Tyra) or octopamine (Octo), molecules related in structure to DA, did not elicit responses when tested at concentrations up to 300 μM , indicating that none of these biogenic amines directly activate GABA ρ 1 (data not shown). Interestingly, when each of these compounds was coapplied with 3.5 μM GABA, a concentration near the EC₅₀ for GABA, we observed a reversible decrease of GABA currents that was dependent on the concentration of the amine tested (Figure 1A). DA was the most potent inhibitor of the GABA ρ 1 current (IC₅₀ of $210 \pm 11.2 \mu\text{M}$; $n = 9$) followed by 5-HT (IC₅₀ of $340 \pm 22.3 \mu\text{M}$; $n = 9$), Tyra ($550 \pm 10.3 \mu\text{M}$; $n = 9$), and Octo ($750 \pm 11.7 \mu\text{M}$; $n = 9$) (Figure 1B). Apomorphine, a nonselective agonist of DA receptors and 5-carboxamido-tryptamine, a nonselective full agonist of 5-HT receptors, did not inhibit GABA ρ 1 responses. The reduction of GABA ρ 1 responses by DA and 5-HT was not correlated with changes of the activation (τ_{act}) or deactivation times (τ_{deac}) of the GABA

current. The τ_{act} and τ_{deac} for GABA alone were 1.08 ± 0.5 s and 2.22 ± 0.9 s, respectively. The τ 's were not modified when we coapplied either 210 μM DA (1.18 ± 0.3 s and 2.17 ± 0.7 s for τ_{act} and τ_{deac} ; $P > 0.05$) or 310 μM 5-HT (1.91 ± 0.5 s and 2.31 ± 0.7 s; $P > 0.05$). In contrast, GABA $_A$ receptors ($\alpha 1\beta 2\gamma 2$) activated by 13 μM GABA (equal to the EC₅₀) were not affected by any of the biogenic amines at the range of concentrations tested (10 μM to 1 mM). In this case, GABA was applied every 10–15 min to avoid the desensitization of the receptor (Figure 1C).

Even though DA, Tyra, and Octo share similar core structures, DA was the most potent inhibitor of GABA ρ 1, indicating that the two adjacent hydroxyl groups in the phenolic ring of DA form an important moiety that enhances the affinity of DA for GABA ρ 1. The absence of inhibitory modulation by apomorphine and 5-carboxamido-tryptamine suggests that the binding site for DA and 5-HT on the GABA ρ 1 receptor may not be structurally similar to the agonist binding site on the DA and 5-HT receptors.

To determine whether DA and 5-HT are competing with GABA for the same binding site, we constructed concentration–response curves for GABA in the presence of different concentrations of DA or 5-HT. Figures 2 and 3 show that DA and 5-HT shifted the GABA curves to the right in a nonparallel manner ($n = 9$ each). Moreover, DA and 5-HT caused a nonsurmountable antagonism of the response to GABA, indicating a noncompetitive antagonism. Linear regressions of Schild plots for DA and 5-HT yielded straight lines with mean slopes different from 1. The slopes of the Schild plots were 2.7

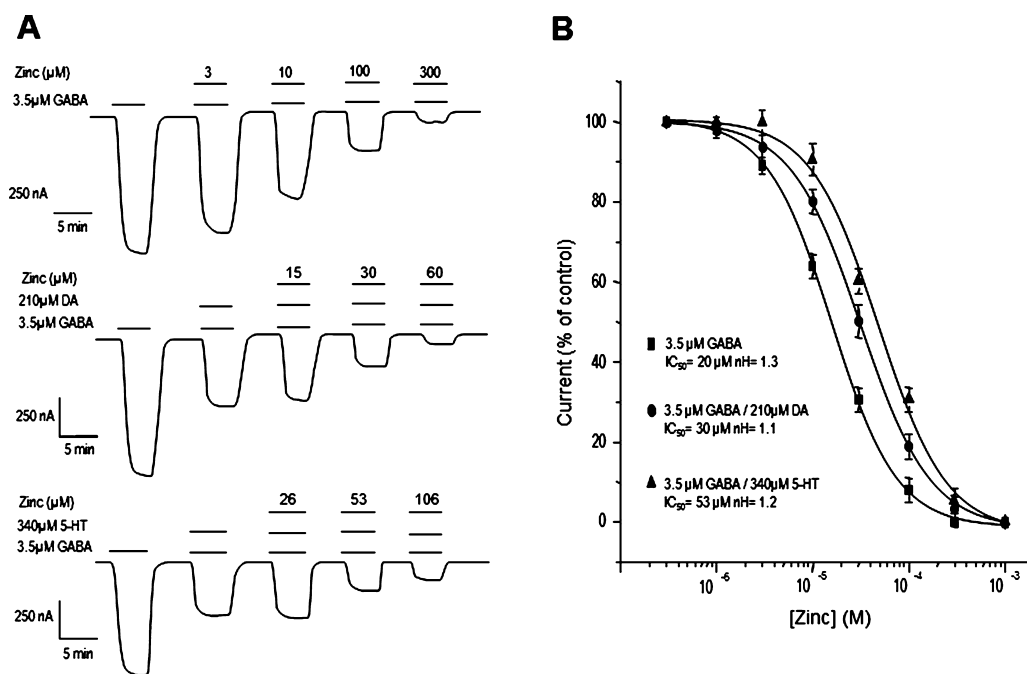


Figure 4. Effect of the GABA ρ receptor antagonist TPMPA on the GABA-elicited currents modulated by DA. (A) Sample records showing that TPMPA effectively and reversibly blocked the currents generated by 3.5 μM GABA and those modulated by 105, 210, and 750 μM DA. Note that the coapplication of DA concentration, higher than 105 μM , and TPMPA enhanced the inhibitory effect. (B) TPMPA antagonism on currents elicited by coapplication of GABA and either DA at 105, 210, and 750 μM . Data points are the means \pm SEM from 6 oocytes ($n = 6$) from 5 frogs ($N = 3$).

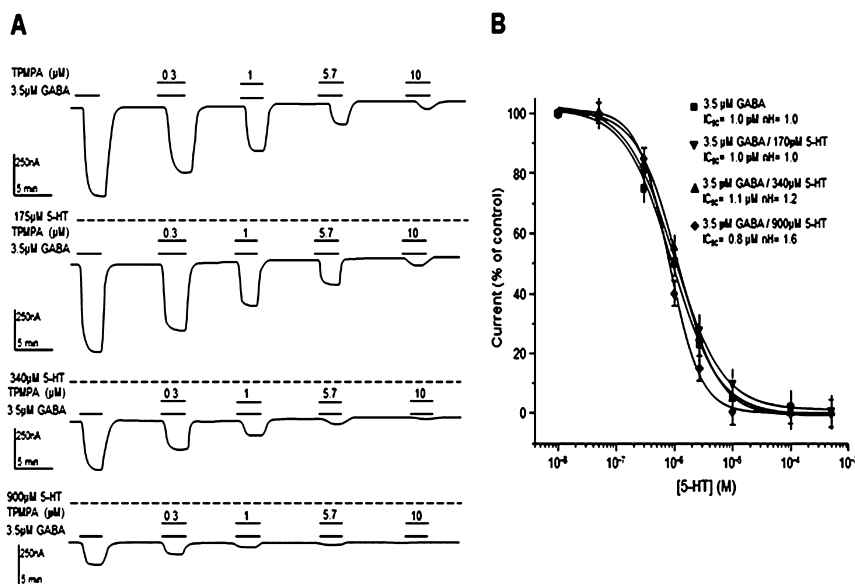


Figure 5. Effect of the GABA ρ receptor antagonist TPMPA on the GABA-elicited currents modulated by 5-HT. (A) Sample records showing that TPMPA effectively and reversibly blocked the currents generated by 3.5 μM GABA and modulated by 175, 340, and 900 μM 5-HT. (B) TPMPA antagonism on currents elicited by coapplication of GABA and either 5-HT at 175, 340, and 900 μM . The currents were adjusted to the amplitude of the maximum current for each curve. Data points are the means \pm SEM from 6 oocytes ($n = 6$) from 5 frogs ($N = 5$).

± 0.3 for DA and 6.1 ± 1.8 for 5-HT; both values confirmed that DA and 5-HT do not compete with GABA for the same binding site on the receptor. The intercepts of the line with the abscissa (pA_2 value) were 3.7 ± 0.9 for DA and 4.7 ± 0.3 for 5-HT, corresponding to apparent equilibrium dissociation constants (K_B) of $4.8 \pm 0.5 \mu\text{M}$ and $5.2 \pm 0.9 \mu\text{M}$, respectively. Because the inhibition was noncompetitive, we used the method of Gaddum et al.²⁵ to calculate the K_B , and the values obtained were 3.04 and $4.9 \mu\text{M}$ for DA and 5-HT, respectively;

both values were similar to those estimated by Schild plots. The inhibitory effects of DA or 5-HT on the GABA currents were independent of the concentrations of GABA used to activate the channel (range of EC_{25} to EC_{75}) (Figures 2C and 3C; $n = 9$ each; $P > 0.05$).

We also analyzed if the negative modulation of DA or 5-HT on GABA currents modified the inhibitory effects of TPMPA, a highly specific competitive antagonist of GABA ρ 1.⁶⁷ As observed in Figure 4, at a concentration of 105 μM , DA did

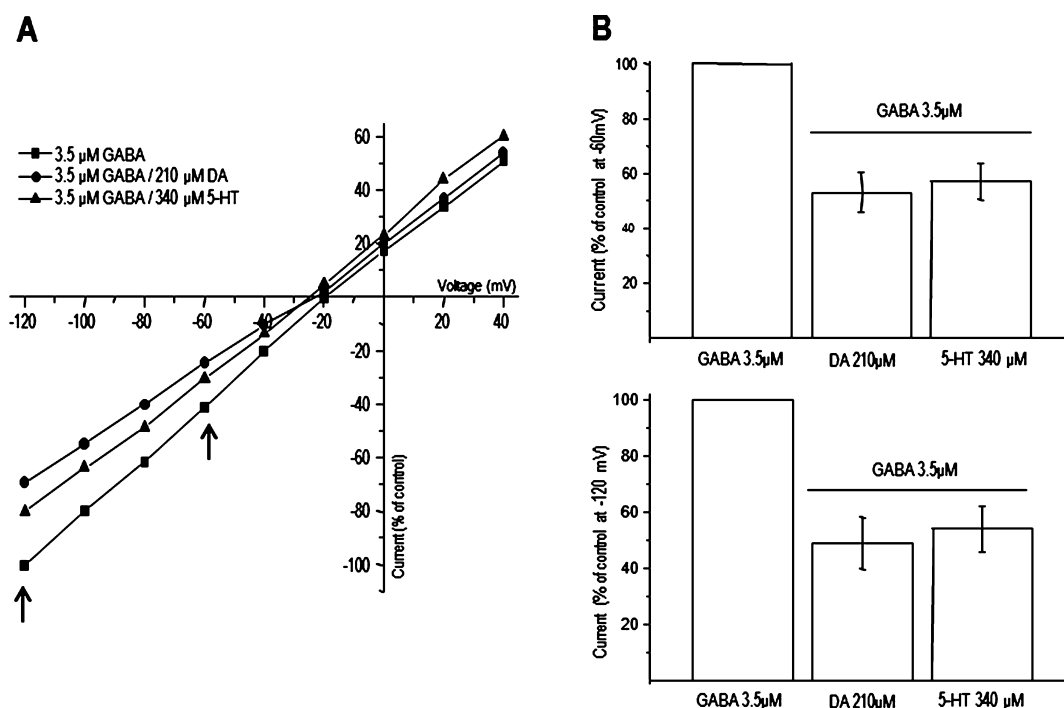


Figure 6. Current–voltage relationships for GABA-elicited currents modulated by DA and 5-HT at indicated concentrations. Note that in all cases the current–voltage relationship is linear and not affected by DA or 5-HT (A). The extent of inhibition is the same at -120 and -60 mV (B). Data points are the means \pm SEM obtained from at least 9 oocytes ($n = 9$) from 5 frogs ($N = 5$).

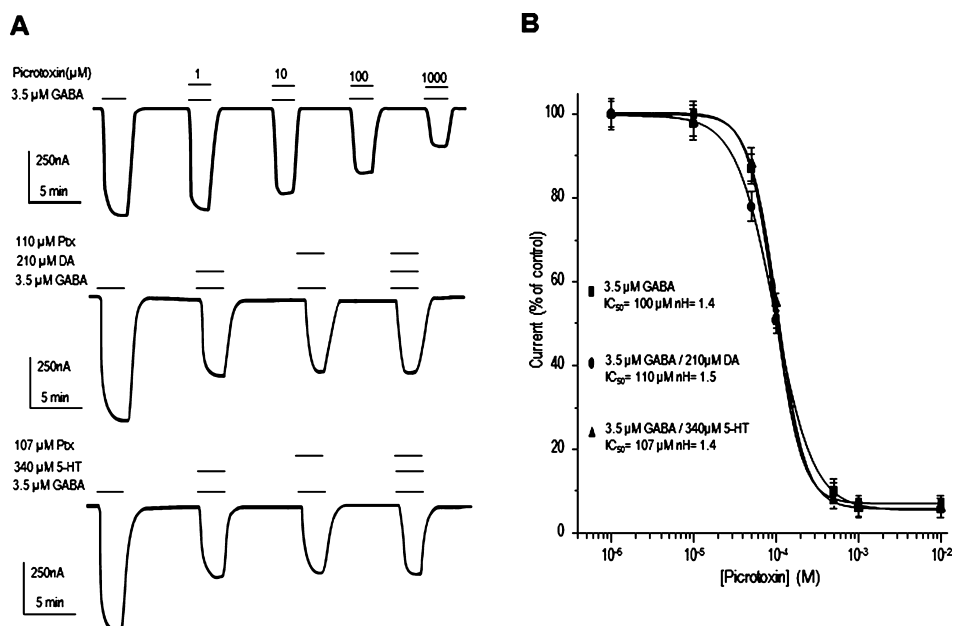


Figure 7. Effect of Picrotoxin on the GABA-elicited currents modulated by DA and 5-HT. (A) Sample records showing that Ptx reversibly blocked the currents generated by 3.5 μM GABA and modulated by 210 μM DA and 340 μM 5-HT. Note that the coapplication of the biogenic amines and Ptx did not enhance the inhibitory effect. (B) Picrotoxin antagonism of the currents elicited by coapplication of GABA and either DA or 5-HT. The currents were normalized to the maximum amplitude elicited by GABA+DA or GABA+5HT in absence of Ptx. Data points are the means \pm SEM from at least 8 oocytes ($n = 8$) from 5 frogs ($N = 5$).

not affect the inhibitory activity of TPMPA; however, at higher concentrations, DA gradually increased the slope of the TPMPA inhibitory curve ($n_H = 0.8 \pm 0.08$, 0.9 ± 0.08 , 1.3 ± 0.03 , and 2.0 ± 0.02 for GABA alone and with 105, 210, and 750 μM DA, respectively), thus indicating a cooperative effect between TPMPA and DA. The IC_{50} for TPMPA did not change with the different concentrations of DA ($IC_{50} = 1.1 \pm$

0.07 , 1.1 ± 0.09 , 1.0 ± 0.09 , and $0.7 \pm 0.1 \mu\text{M}$ for GABA alone and with 105, 210, and 750 μM DA, respectively; $P > 0.05$). The additive antagonism of TPMPA and DA indicates that these two compounds bind to different sites within GABA ρ 1. 5-HT also gradually increased the Hill coefficient for the TPMPA antagonist effect; however, its effects were not as strong as those of DA ($n_H = 1 \pm 0.05$, 1 ± 0.05 , 1.2 ± 0.07 , and $1.6 \pm$

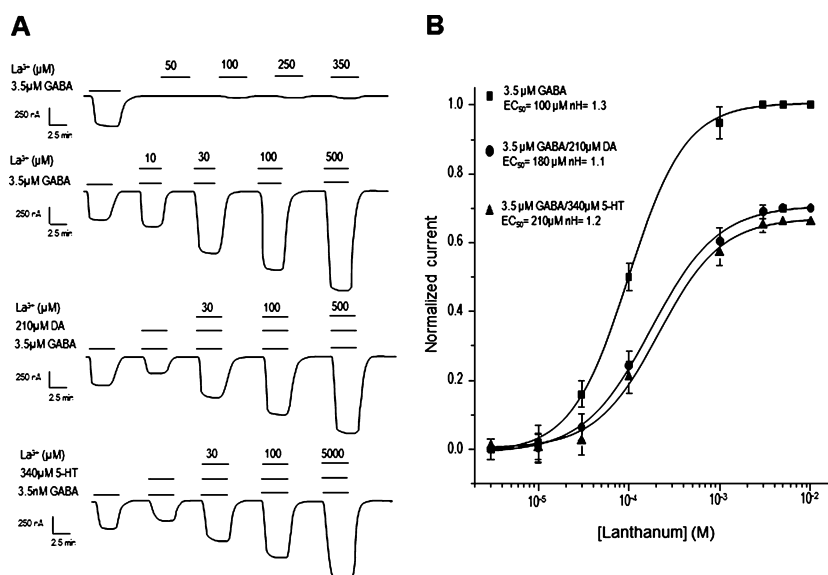


Figure 8. Lanthanum modulation. (A) Sample records illustrating the activation of GABA ρ 1 receptors and the potentiation of GABA-currents by La $^{3+}$ and their modulation by DA and 5-HT. (B) La $^{3+}$ concentration–response relations of the currents generated by 3.5 μ M GABA and modulated by 210 μ M DA and 340 μ M 5-HT. Observe the blocking effect of DA and 5-HT on the GABA-elicited current in presence of La $^{3+}$. The currents were normalized to the amplitude of that elicited by 3.5 μ M GABA alone. Data points are the means \pm SEM from at least 8 oocytes ($n = 8$) from 5 frogs ($N = 5$).

0.09 for GABA alone and with 170, 340, and 900 μ M 5-HT, respectively) (Figure 5). 5-HT did not modify the IC $_{50}$ for TPMPA at any of the concentrations tested (IC $_{50} = 1 \pm 0.09$, 1 ± 0.04 , 1.1 ± 0.1 , and 0.8 ± 0.1 μ M for GABA alone and with 179, 340, and 900 μ M 5-HT, respectively; $P > 0.05$).

Available evidence indicates that DA may interact with other ligand-gated ion channels by blocking the ion pathway (e.g., glutamate NMDA receptors expressed in oocytes or present in neurons grown in culture^{26,27}). Since the voltage dependence of the antagonism is a shared characteristic among several compounds that bind inside the pore,²⁷ we explored whether the effects of DA and 5-HT were voltage dependent. Current–voltage relationships were constructed for the activation of the receptors with 3.5 μ M GABA alone and during coapplication of GABA with DA or 5-HT IC $_{50}$ values (210 and 340 μ M, respectively). As shown in Figure 6A, in all cases, the current–voltage relationships were linear within the range explored (–120 to +40 mV), indicating that the effects of DA and 5-HT were voltage independent and inhibited equally at –60 and –120 mV (Figure 6B). None of these biogenic amines changed the ion selectivity of the channel, as suggested by their inversion potential which was -23 ± 1.9 mV ($n = 9$) for GABA and -25 ± 0.8 mV ($n = 9$) or -28 ± 0.6 mV ($n = 9$) in the presence of DA or 5-HT, indicating that chloride remains as the main ion flowing through the GABA-gated channel. To further explore a possible ion pore blockade mechanism, we analyzed if DA and 5-HT affect the blocking of GABA-currents by picrotoxin (Ptx), a noncompetitive antagonist of GABA receptors whose binding site is in the ion pathway.²⁸ As shown in Figure 7, the inhibitory actions of Ptx were not affected by DA or 5-HT. We did not observe any synergic inhibitory effect when Ptx was coapplied with DA or 5-HT. One possible explanation for this is that Ptx binding to GABA ρ 1 alters the conformation such that the binding site for the biogenic amines is not exposed. The IC $_{50}$'s and Hill coefficients for Ptx were 100 ± 1.0 μ M and 1.4 ± 0.9 for GABA ($n = 5$), 100 ± 1.5 μ M and 1.5 ± 0.8 for GABA+DA ($n = 6$,

and 107 ± 0.9 μ M and 1.4 ± 0.7 for GABA+5-HT ($n = 5$ –6 each). These results contrast with those observed in NMDA receptors, in which DA, 5-HT, and even Tyra clearly interact with the narrowest region of the channel pore of the receptor.^{26–28} Thus, according to the competition assays, competition with Ptx, and current–voltage relationships, the binding site for DA and 5-HT in GABA ρ 1 is different from the GABA binding site, and it is probably not located inside the channel pore. Future point-mutation studies will precisely determine if DA and 5-HT bind inside the pore of the channel.

La $^{3+}$ acts on GABA $_A$ receptors at a site different from those used by barbiturates, benzodiazepines, Ptx, or Zn $^{2+}$, and in some biological preparations La $^{3+}$ induces a Cl $^-$ current by directly activating GABA $_A$ receptors.^{31–36} It was previously shown that La $^{3+}$ and other lanthanides positively modulate GABA ρ 1 receptors,³⁷ suggesting the existence of a La $^{3+}$ allosteric site in these receptors.³⁸ In our experiments, the application of 100 μ M La $^{3+}$ alone induced small inward currents in oocytes that expressed GABA ρ 1 (Figure 8A). This current was not observed in noninjected oocytes, suggesting a direct activation of the GABA ρ 1 receptors by La $^{3+}$. Lanthanum also increased, in a concentration-dependent manner, the amplitude of the currents elicited by GABA alone. The potentiation of GABA-currents by La $^{3+}$ was not prevented by the negative modulation of DA or 5-HT (Figure 8B); nevertheless, the magnitude of the GABA-induced currents modulated by DA or 5-HT in presence of La $^{3+}$ was smaller than the control. The presence of the amines reduced the potency of La $^{3+}$, as observed by the right-shift of the dose response from 100 ± 1.8 μ M for GABA alone ($n = 5$) to 186 ± 1.1 μ M for GABA + DA ($n = 5$; $P > 0.05$, different than the control) and 210 ± 1.2 μ M for GABA + 5-HT modulated currents ($n = 5$; $P > 0.05$, different than the control). Furthermore, the negative modulation of DA and 5-HT was not overcome by larger concentrations of La $^{3+}$ (Figure 8B). No changes in the Hill coefficients were observed (1.3 ± 0.1 , 1.1 ± 0.07 , and 1.2 ± 0.08 , for GABA alone, GABA+DA, and GABA+5HT

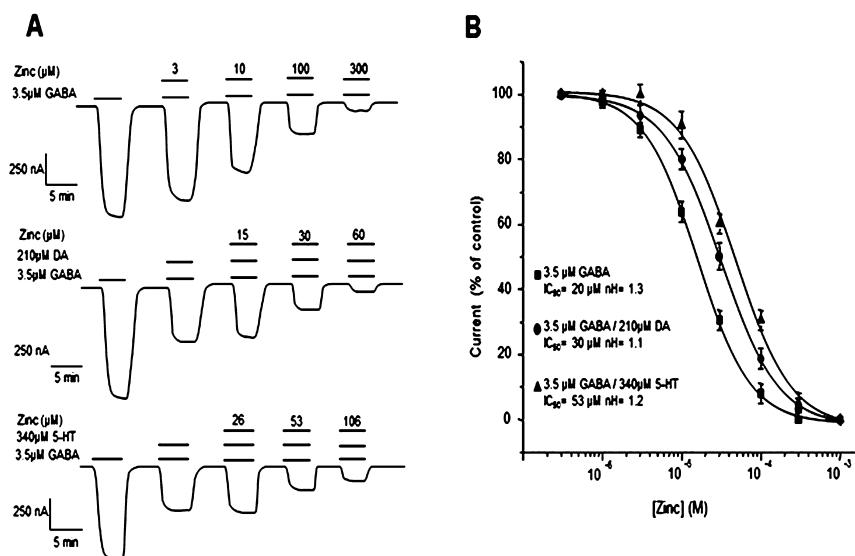


Figure 9. Zinc inhibition. (A) Inhibition of the currents elicited by GABA alone and modulated by DA and 5-HT. The coapplication of either DA or 5-HT with Zn^{2+} enhances the inhibitory effect. (B) Concentration–response relationships of the currents elicited by 3.5 μM GABA and modulated by 210 μM DA and 340 μM 5-HT. Note the displacement of the curves in the presence of DA or 5-HT. The currents were normalized to the amplitude of that elicited by GABA+DA or GABA+5HT in absence of Zn^{2+} . Data points are the means \pm SEM from at least 7 oocytes ($n = 7$) from 5 frogs ($N = 5$).

respectively). Since these data suggest a noncompetitive interaction between the amines and La^{3+} , we investigated whether DA and 5-HT affected the inhibition of the receptor by Zn^{2+} , which apparently binds to a different allosteric site than La^{3+} .³⁹

The GABA-currents negatively modulated by DA and 5-HT were further inhibited by Zn^{2+} . The additive inhibitory effects of Zn^{2+} and either DA or 5-HT reached about 80% total inhibition in both cases (Figure 9A). Interestingly, DA and 5-HT had different effects on the sensitivity of GABA-currents to Zn^{2+} . In Figure 9B, it is shown that DA increased the sensitivity of GABA-currents to inhibition by Zn^{2+} . In contrast, 5-HT right-shifted the concentration–response curve of Zn^{2+} . The concentration–response relationship for Zn^{2+} gave IC_{50} values of $30 \pm 0.7 \mu M$ ($n = 7$) for GABA alone, $20 \pm 0.5 \mu M$ ($n = 7$) for GABA+DA, and $53 \pm 0.8 \mu M$ for GABA+5-HT ($n = 7$) ($P > 0.05$, different to the control). No significant differences among the Hill coefficients were observed: 1.3 ± 0.08 , 1.1 ± 0.1 , and 1.2 ± 0.09 , for GABA, GABA+DA, and GABA+5-HT, respectively (Figure 9B). One possible explanation for the opposite effects of DA and 5-HT on GABA ρ 1 sensitivity to Zn^{2+} is that the allosteric modulation could occur via different structural rearrangements. Interestingly, competition between Zn^{2+} and DA for the same binding site has been described in the DA type 1 and 2 receptors expressed in HEK and CHO cells.^{40–42}

In conclusion, we found that DA and 5-HT directly modulate homomeric GABA ρ 1 receptors but not GABA $_A$ receptors. Since the concentrations of DA and 5-HT in the retina vary between 0.9 and 470 μM and 0.1 and 590 μM ,^{32,43–45} respectively, both of which are within the range of modulation we found for GABA ρ 1, it is plausible that these interactions occur in the retina. Such possibilities will be explored in future studies.

METHODS

Expression of Human GABA $_A$ Receptors. All the animals were handled in accordance with the guidelines of the National Institute of

Health Guide for Care and Use of Laboratory Animals, and with the approval of the Institutional Animal Care and Use Committee of the National University of Mexico. *X. laevis* frogs were anesthetized with 0.17% 3-aminobenzoic acid methyl ester (MS-222) for 20–30 min. Follicles were manually removed, enzymatically defolliculated (with 0.3 $\mu g/\mu L$ collagenase type I at room temperature for 45 min), and then kept at 16 $^{\circ}C$ in Barth's medium: 88 mM NaCl, 1 mM KCl, 0.33 mM $Ca_2(NO_3)_2$, 0.41 mM $CaCl_2$, 0.82 mM $MgSO_4$, 2.4 mM $NaHCO_3$, 5 mM HEPES, pH 7.4, containing 0.1 mg/mL gentamicin sulfate. The next day, 50 nL (0.5 $\mu g/\mu L$) of human GABA ρ 1 mRNA or 18 nL (0.5 $\mu g/\mu L$) of GABA $_A$ ($\alpha 1\beta 1\gamma 2$) cDNA were injected in the equator or in the nucleus of the oocyte, respectively. The electrophysiological recordings were obtained 3–5 days after injection.

Voltage-Clamp Recordings. The membrane currents elicited by the agonists were recorded using the two-microelectrode voltage-clamp technique.⁴⁶ Oocytes were placed in a 500 μL chamber, impaled with two glass microelectrodes filled with 3 M KCl (0.5–2.5 M Ω) and clamped at -60 mV. To obtain the equilibrium membrane potential of the agonist transmitter action, current–voltage relationships were constructed by stepping the oocyte's membrane potential from -60 to -120 mV for 1 s and then from -120 to $+40$ mV (in 20 mV steps) in the absence or presence of GABA, DA, 5-HT, Tyra, Octo, the dopaminergic agonist apomorphine, or the serotonergic agonist 3-carboxamido-tryptamine. All recordings were done at room temperature (20–23 $^{\circ}C$) in a chamber continually perfused (5–10 mL/min) with frog Ringer solution: 115 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 5 mM HEPES, pH 7.4. All drugs were purchased from SIGMA. The stock solution of GABA (1 M) was stored frozen, and fresh dilutions were used for the experiments. All monoamines were prepared the day of the experiment by dissolving them directly in frog Ringer solution. The pH of all solutions was adjusted to 7.4.

Data Analysis. Results are reported as mean \pm SEM of the values obtained from several cells. Data from each experiment were collected from at least seven oocytes. Agonist concentration–response curves were constructed by measuring the maximum response evoked by each agonist concentration. The half-maximal concentration (EC_{50}) and Hill coefficient (nH) were estimated for each curve by fitting the data to the logistic type equation (Origin 6.0, Northampton, MA): $A = A_{max}/(1 + 10^{[\log EC_{50} - [\log [agonist] nH]]})$. The half-inhibitory concentration (IC_{50}) of DA, 5-HT, TPMPA, or Zn^{2+} was estimated by fitting the following equation: $A = A_{max}/(1 + 10^{[\log [agonist] - \log IC_{50}]})$. To determine the time constants for the activation (τ_{act}) and deactivation (τ_{deac}) of

GABA-current responses, a decay function of the form $I(\tau) = \exp(-t/\tau_d) + C$, where I is the current and t is time, was fit to the experimental data (Origin 6.0 software; Northampton, MA). Differences between groups were statistically analyzed by ANOVA and a Tukey-Kramer post-test. Differences were considered significant at the level $P > 0.05$. The pA_2 values ($-\log$ of the molar concentration of agonist that reduces the agonist EC_{50} by a factor of 2) for DA and 5-HT were determined from Schild plots using GABA as agonist (GraphPad Prism 4). The concentration ratio (the ratio between the EC_{50} values for GABA in the presence and absence of an agonist) at different antagonist concentrations for the different GABA/antagonist pairs were plotted in a Schild diagram using regression analysis, and the pA_2 value was obtained from the intercept of the regression line with the abscissa. Since DA and 5-HT showed a noncompetitive antagonistic effect on GABA ρ 1 receptors, the affinity was calculated by the method of Gaddum.²⁵ Logistic equations of the form $I(x) = I_{\min} + (I_{\max} - I_{\min})/[1 + (x/EC_{50})^k]$ were fitted to agonist curves, and the equiactive concentrations at EC_{30} , EC_{40} , EC_{50} , and EC_{60} in the absence and in the presence of different concentrations of DA or 5-HT were calculated from these curves. The reciprocals of the equiactive concentrations are correlated according to the equation $1/[GABA] = (1/[GABA'])(1 + [DA]/K_B) + (\alpha[DA])/(K_A K_B)$, where $[GABA]$ is the concentration of agonist (M) in absence of DA, $[GABA']$ is the equiactive concentration in the presence of a specific concentration of DA (M), α is a modifying term that denotes the change in affinity of one ligand produced by binding of the other, and K_A is the equilibrium dissociation constant for the agonist. Since the reciprocals $1/[GABA]$ and $1/[GABA']$ are linearly correlated, they were plotted, and the linear regression to these data was used to calculate the K_B , using the equation $K_B = [DA]/(\text{slope} - 1)$. Control responses to GABA were obtained before and after each drug application to account for possible shifts in the amplitude of the control current.

AUTHOR INFORMATION

Corresponding Author

*Mailing address: Departamento de Neurobiología Celular y Molecular, Instituto de Neurobiología, Campus UNAM Juriquilla, Queretaro, Qro. México CP 76230. E-mail: ataulfo@unam.mx. Telephone and fax number: (52) 442-238-1064

Author Contributions

A.E.-M. and A.M.-T. purified the plasmids carrying the GABA receptors and synthesized the cRNA, R.M. and L.D.O.P. did the electrophysiology and pharmacology in oocytes, and A.L. and L.D.O.P. did the statistic tests. All the authors participated in the experimental design and writing of the paper.

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Notes

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ABBREVIATIONS

GABA, γ -aminobutyric acid; DA, dopamine; 5-HT, 5-hydroxytryptamine; Tyra, tyramine; Octo, octopamine; Ptx, picrotoxin; TPMPA, (1,2,5,6-tetrahydropyridine-4-yl) methyl-

phosphinic acid; MS-222, ethyl 3-aminobenzoate methanesulfonate; HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; LGIC, ligand-gated ion channels; NMDA, N-methyl-D-aspartate; SCH23390, R-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride; SKF81297, R-(+)-6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol hydrochloride

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