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Effect of Triazine Derivatives on Neuronal Nicotinic Receptors

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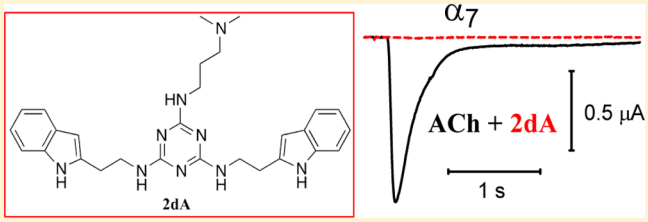
Supporting Information

ABSTRACT: We have characterized the effect of triazine derivatives on neuronal nicotinic receptors expressed in *Xenopus* oocytes. All triazines investigated inhibit the current of $\alpha7$ and $\alpha3\beta4$ neuronal nicotinic receptors elicited by acetylcholine. The effect is concentration dependent, reversible, and noncompetitive. In contrast, some derivatives have a dual effect on $\alpha4\beta2$ receptors, by potentiating the currents at intermediate concentration and causing inhibition at higher concentrations. Triazine derivatives also affect the macroscopic kinetics of the heteromeric receptors $\alpha3\beta4$ and $\alpha4\beta2$ accelerating the rise and decay time course of the currents, but have no significant effect on the kinetics of homomeric $\alpha7$ receptors. Two simple kinetic models are presented. The first reproduces the effects of different concentrations of triazines both on the peak currents and on the macroscopic kinetics of $\alpha7$ with a simple inhibitory result. The second model describes the behavior of $\alpha4\beta2$ receptors involving a more complex dual action.



KEYWORDS: *Neuronal nicotinic receptors, triazine derivatives, α7, α4β2, ionic currents, kinetic model*

Neuronal nicotinic receptors (NNRs) are a family of ligand gated ion channels widely distributed in both the peripheral (PNS) and central (CNS) nervous systems and naturally activated by the neurotransmitter acetylcholine (ACh). These receptors play an important role in different physiological and neuropathological states, including Parkinson's and Alzheimer's diseases, pain, anxiety, and tobacco dependency.[1] NNRs are composed of five homologous subunits symmetrically arranged around the ion pore, and there are several types of receptors depending on its subunit composition; each type has its own pharmacological and functional characteristics. NNRs are the targets of many substances that are being explored for the treatment of the diseases mentioned above.[2]

Some triazine derivatives (Tzs) have been shown to have an effect on nicotinic receptors, for example, the drug lamotrigine is a phenyltriazine derivative used as an anticonvulsant drug that is useful for alleviating epilepsy and bipolar disorder. Lamotrigine has agonist effect on adult muscle nicotinic acetylcholine receptors[3] and blocks $\alpha4\beta2$ neuronal nicotinic receptors.[4] Some Tzs used in this study have also been shown to act as an open channel blocker in transient receptor potential vanilloid 1 channels.[5]

In the present work, a group of 2,4,6-trisubtituted-1,3,5-triazines (Figure 1) have been synthesized and tested as antagonists of $\alpha7$ (present in PNS and CNS), $\alpha4\beta2$ (predominant subtype in CNS), and $\alpha3\beta4$ (predominant subtype in PNS) acetylcholine receptors. The triazines were substituted at the R_1 position with different amines containing electron donating and electron withdrawing substituents linked to aromatic groups in order to evaluate the effect of these moieties. The importance of the alkyl amine nature was studied at the R_2 position as well.

RESULTS AND DISCUSSION

Triazine 2dA Inhibits NNRs and Serotonin Receptors but not Glycine Receptors. Of all the triazines studied, **2dA** was the one that inhibited NNRs most effectively. As shown in Figure 2A, the application of 3 μM **2dA** greatly decreased the currents elicited by 200 μM ACh in $\alpha7$, $\alpha4\beta2$, and $\alpha3\beta4$ NNRs and that elicited by 3 μM serotonin in the 5-HT_{3A} receptor. In contrast, the effect was very small on the currents stimulated by 100 μM glycine in the glycine $\alpha1$ receptor. The concentrations of agonist used for each receptor were close to the EC_{50} to allow for a possible increase or decrease of the current produced by the Tzs. Figure 2B shows the statistical results of the effect of **2dA** on the peak currents of these receptors. The maximum effect was observed on $\alpha7$ receptors with peak current reduced to 2% of the control value, followed by 5-HT_{3A} reduced to 6%. However, the heteromeric receptors $\alpha4\beta2$ and $\alpha3\beta4$ were only reduced to 15%, and glycine receptors conserved more than 90% of the original current.

Besides the effect on the peak current, the time course of the current was also altered by **2dA** in the heteromeric NNRs, as can be appreciated in the current records of Figure 2A. Statistical analysis reveals that the 10−90% rise time (in

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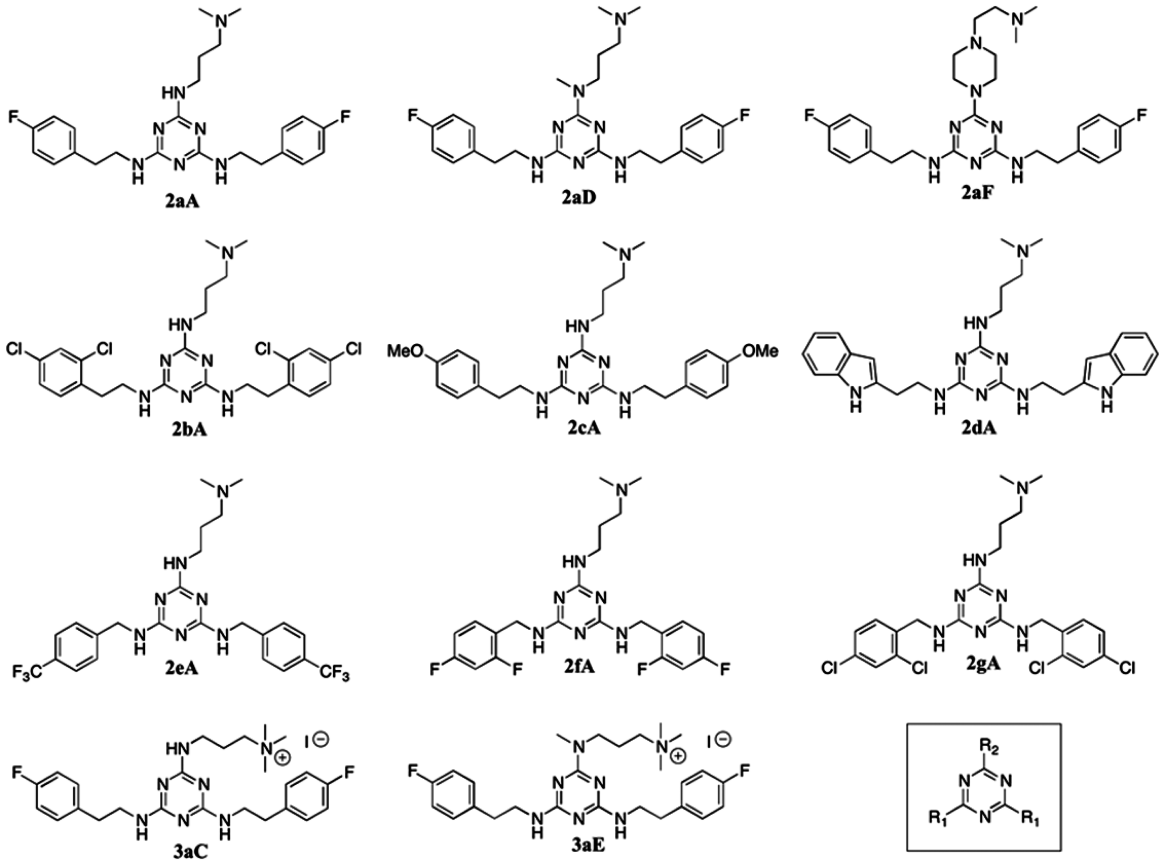

Figure 1. Structure of the triazine derivatives used in this study. The general formula of Tzs is shown in the lower right corner.

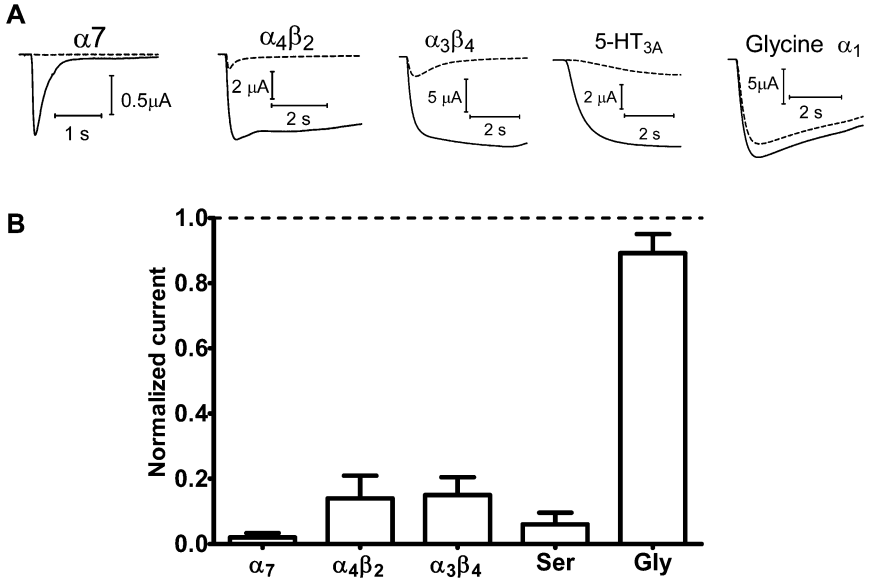

Figure 2. Representative current records and statistical analysis of the effect of **2dA** on NNR, serotonin receptors, and glycine receptors. (A) Current records obtained in control (only agonist present, solid lines) and **2dA** application conditions (agonist plus 3 μM **2dA** was applied in all cases, dashed lines). ACh (200 μM) was applied to $\alpha7$, $\alpha4\beta2$, and $\alpha3\beta4$ receptors, 3 μM serotonin to 5-HT_{3A} receptors, and 100 μM glycine to glycine receptors. (B) Mean and SE values of the normalized peak current obtained in the presence of **2dA**.

milliseconds) for $\alpha3\beta4$ currents decreased from 720 ± 30 (73) in control conditions to 204 ± 8 (6) in the presence of 3 μM **2dA**, and for $\alpha4\beta2$, the rise time decreased from 337 ± 30 (73) to 67 ± 9 (6) with **2dA**, both differences being highly significant ($p < 0.001$). The 100−50% decay time was also affected for those two receptors, decreasing from values larger than the pulse duration (4000 ms) to 806 ± 43 (5) ms for $\alpha3\beta4$ and 188 ± 26 (6) ms for $\alpha4\beta2$. In contrast, the macroscopic kinetics of the currents of glycine and serotonin receptors was not significantly affected. Finally, the residual $\alpha7$ current after block by **2dA** was too small to reliably measure the time course.

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The effect of Tzs on the currents was slowly reversible. After 2 min of washing with the normal perfusion solution, the current recovered to most of its original value.

For the rest of the study we concentrated on the effect of Tzs on NNRs.

Most Triazines Block NNRs. Figure 3 shows the effect of 3 μM triazines on the magnitude of NNR peak currents elicited

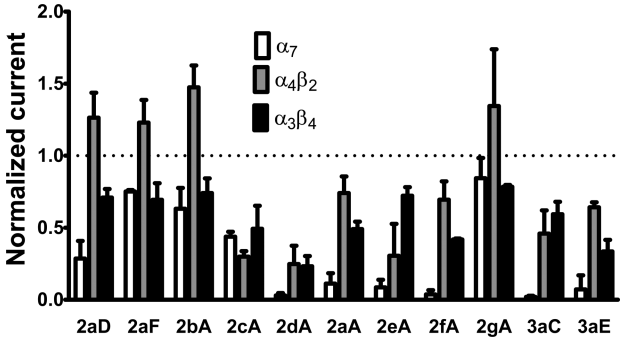

Figure 3. Normalized current of $\alpha7$, $\alpha3\beta4$, and $\alpha4\beta2$ receptors elicited by ACh in the presence of 3 μM of different Tzs. Control values of peak inward currents (in μA) were $\alpha7$, 1.22 ± 0.07; $\alpha4\beta2$, 5.05 ± 0.39; and $\alpha3\beta4$, 11.85 ± 0.50.

by ACh. $\alpha7$-Receptor is the most sensitive; its peak current is reduced by all triazines studied, and in general, the least sensitive is $\alpha4\beta2$. Furthermore, as stated above, the most effective triazine in blocking NNRs was **2dA**. From the structural point of view, triazine **2dA** is the only one that bears a nitrogen heteroaromatic fragment (indole moiety) as part of the R_1 substituents. All the other triazines tested contain only aromatic residues. Thus, the protonable nitrogen atom present in the indole residues can contribute decisively to the higher antagonist activity elicited by **2dA** in NNRs, in particular in $\alpha7$. It is worth noting that triazines bearing a formal positive charge as a quaternary ammonium moiety at the nonaromatic residue R_2 (cf. compounds **3aC** and **3aE**) exhibit also a remarkable effect on this nicotinic receptor, although the effect is weaker on the heteromeric receptors $\alpha4\beta2$ and $\alpha3\beta4$. On the other hand, the presence of aromatic residues bearing electron-donating groups, such as OCH_3 in **2cA**, causes a higher antagonist effect in comparison with triazines bearing electron-withdrawing substituents (**2aD**, **2aF**, **2bA**, or **2gA**), with the exception of **2eA** and **2fA** only on $\alpha7$ receptor. Noticeably, these compounds have one carbon less as linker between the aromatic residue and the amino group attached to the triazine ring.

Curiously, in $\alpha4\beta2$ receptors the effect of triazines was dual: some reduced the current as with the other NNRs, but more interestingly, some Tzs enhanced the current. This enhancement effect was studied in more detail in other experiments.

Figure 4 shows the corresponding effect of 3 μM triazines on the rise time course of NNR currents elicited by ACh. It is notable to observe that $\alpha7$ macroscopic kinetics is not significantly affected by Tzs in all cases where the residual current is large enough to be measured with precision. In contrast, the effect of Tzs on the kinetics of macroscopic currents of heteromeric receptors were all accelerated, with rise times reduced to half the control values or less in most cases for $\alpha3\beta4$ and with a slightly smaller effect for $\alpha4\beta2$. Likewise, the decay time from 100% to 50% of peak current was not significantly changed in $\alpha7$ by Tzs in all cases where the

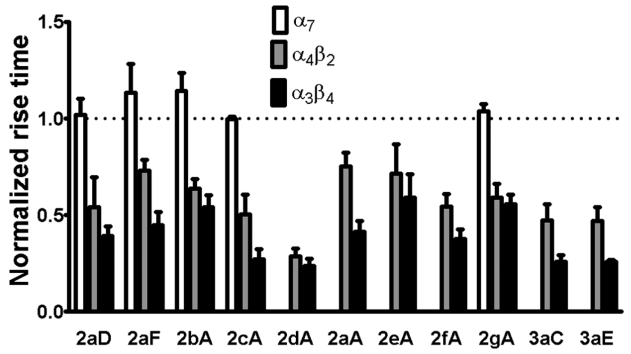

Figure 4. Normalized 10–90% rise time of $\alpha7$, $\alpha3\beta4$, and $\alpha4\beta2$ receptors in the presence of different Tzs at 3 μM. Control values of rise times (in ms) were $\alpha7$, 44.5 ± 0.8; $\alpha4\beta2$, 450 ± 17; and $\alpha3\beta4$, 860 ± 23.

remaining current could be accurately measured (i.e., for Tzs **2aD**, **2aF**, **2bA**, **2cA**, and **2gA**). Moreover, in the few cases where decay times could be measured in control conditions for heteromeric receptors, the effect of Tzs was very large (**2eA** reduced the decay time of $\alpha3\beta4$ by a factor of 0.57 ± 0.06; similarly **2dA** and **3aE** reduced the decay time of $\alpha4\beta2$ by a factor of 0.11 ± 0.02 and 0.40 ± 0.02, respectively), and this is an underestimate of the effect since most of the currents of heteromeric receptors did not decay down to 50% of the peak value during the control pulse. Control values of decay times were $\alpha7$, 139 ± 3.0; $\alpha4\beta2$, 2100 ± 160; and $\alpha3\beta4$, ≥3000 ms.

The Effect of Triazines Is Dose-Dependent. Figure 5 shows dose–response curves of the effect of three different Tzs on peak currents of NNRs. IC_{50} values are between 0.8 and 4.5 μM in all cases. Compound **2dA** is the most potent inhibitor on $\alpha7$, and also the inhibition curves have a Hill coefficient greater than 3 indicating a high degree of cooperativity. In contrast, the sensitivity of heteromeric NNRs is smaller with Hill coefficients closer to 1 or even smaller as in the case of **2cA**, for which it is 0.6 ± 0.2 in both $\alpha3\beta4$ and $\alpha4\beta2$ receptors. Also, the effect of **2bA** on $\alpha4\beta2$ is not a simple inhibition and will be explained later. These experiments also showed that high doses of Tzs produce a strong inhibition of the current that takes a long time to recover, although no attempt was made to systematically measure the recovery time as a function of the concentration.

The Effect of Tzs Is Not Competitive. Figure 6 shows the effect of **2dA** 1 μM on dose–response curves of ACh for NNRs. In all cases, there is a reduction of I_{max} (of about 50%) in the presence of **2dA** suggesting a noncompetitive mechanism of action of Tzs on NNRs. Furthermore, in $\alpha7$ and $\alpha3\beta4$, there is a right shift of the apparent EC_{50} that increases by a factor of 1.7 and 2.8, respectively. In contrast, the right shift in EC_{50} is not significant for $\alpha4\beta2$. The Hill coefficients do not change either in $\alpha3\beta4$ and $\alpha4\beta2$, and the comparison was not possible in $\alpha7$ because the error of the fit in this parameter is very large, probably because of the initial decrease of the current observed at high concentrations of ACh in the presence of **2dA**. It is worth noting that ACh dose–response curves of $\alpha4\beta2$ receptors expressed in *Xenopus* oocytes have been reported to be better fitted by a biphasic Hill equation. This is due to the expression of two different populations of receptors, one with high (HS) and the other with low (LS) sensitivity to ACh, reflecting a different stoichiometry: $(\alpha4)_2(\beta2)_3$ and $(\alpha4)_3(\beta2)_2$, respectively. This effect was observed even when the subunit cDNAs for $\alpha4$ and $\beta2$ were injected in equal proportions.[6] In contrast, our results (also obtained by injecting the oocytes

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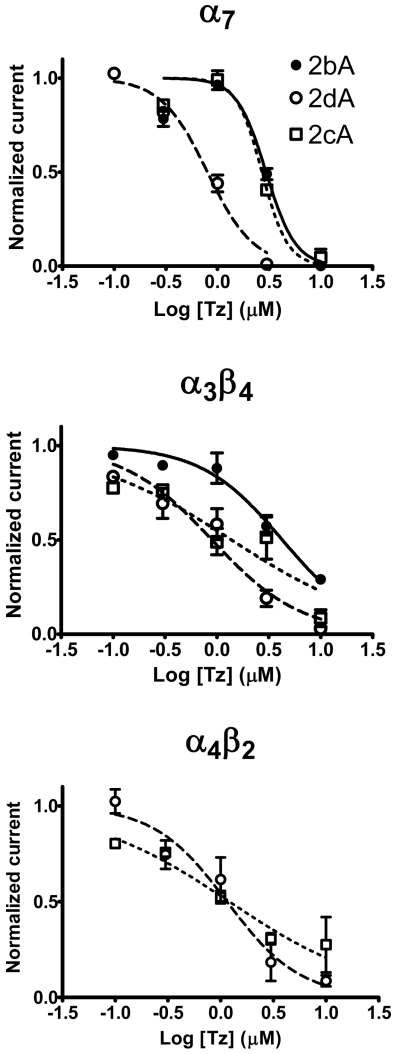

Figure 5. Dose−response curves of the inhibitory effect of Tzs on peak currents elicited by ACh in NNR. Curves are fits to the modified Hill equation with the following parameters (IC_{50} in μM, *nH*): α7, **2bA** (3.0 ± 0.2, 3.0 ± 1.5), **2dA** (0.8 ± 0.1, 1.9 ± 0.3), **2cA** (2.7 ± 0.3, 3.5 ± 2.1); $\alpha3\beta4$, **2bA** (4.3 ± 0.6, 1.1 ± 0.2), **2dA** (0.9 ± 0.2, 1.0 ± 0.2), **2cA** (1.4 ± 0.5, 0.6 ± 0.2)); $\alpha4\beta2$, **2dA** (1.1 ± 0.2, 1.3 ± 0.2), **2cA** (1.2 ± 0.2, 0.6 ± 0.1).

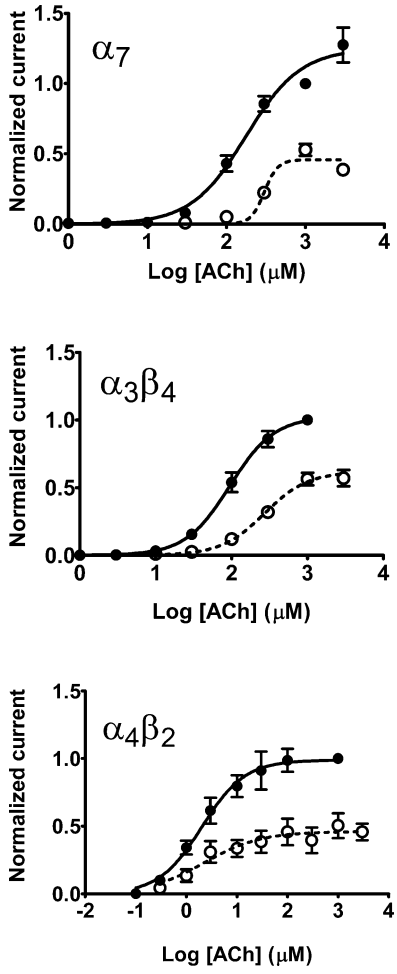

Figure 6. Dose−response curves of peak current elicited by ACh in control conditions (solid dots and line) or ACh in the presence of 1 μM **2dA** (open dots, dashed line). Data have been normalized to the peak current obtained in control conditions with 1 mM ACh. Lines are fits to the Hill equation with the following parameters (I_{max}, EC_{50} in μM, *nH*): α7, control (1.25 ± 0.07, 180 ± 32, 1.3 ± 0.2), with **2dA** (0.46 ± 0.03, 300 ± 21, 5 ± 20); $\alpha3\beta4$, control (1.03 ± 0.05, 95 ± 13, 1.5 ± 0.2), with **2dA** (0.62 ± 0.05, 270 ± 46, 1.5 ± 0.3)); $\alpha4\beta2$, control (0.99 ± 0.05, 2.0 ± 0.5, 1.0 ± 0.2), with **2dA** (0.46 ± 0.05, 2.3 ± 1.7, 0.8 ± 0.4).

with cDNA ratios of 1:1) do not seem to reflect the presence of a mixed population of receptors or at least most of the receptors must belong to the $(\alpha4)_2(\beta2)_3$ stoichiometry. This is because the ACh dose−response obtained can be fitted with a monophasic Hill curve with EC_{50} of 2 μM, close to that reported for the high sensitivity $\alpha4\beta2$ receptors by Moroni et al.[6]

Some Triazines Potentiate $\alpha4\beta2$. As mentioned above, some triazines, that is, **2aD**, **2aF**, **2bA**, and **2gA**, elicit a dual effect on $\alpha4\beta2$ currents: at high concentrations, they reduce the current, but at intermediate concentrations, they potentiate the current. Noticeably, triazines **2aD** and **2aF** contain a trisubstituted nitrogen at the nonaromatic residue attached to the triazine moiety, which can enhance its basicity. On the other hand, in compounds **2bA** and **2gA**, the aromatic residue bears two chloro substituents, and one of them can interact with the NH groups attached to the triazine ring. Beyond these structural considerations, the limited number of compounds that have shown this dual behavior prevents the drawing of conclusive structure−activity relationships. We have studied in more detail the effect produced by **2bA** because it was the triazine that showed the highest potentiating effect. Figure 7A shows the result of applying 3 μM **2bA** on $\alpha4\beta2$ currents elicited by ACh 200 and 2 μM. The average increase of the peak current is approximately 50% in both cases accompanied by a clear change in the kinetics of the current, which becomes faster, both in the rising and in the decaying phases. There is a decrease in rise time of 40%, and the decay time to 50% of the peak is reached in only 1.4 s, whereas the control current does not decay to 50% during the duration of the recording. Figure 7B shows the dose dependence of the effect of **2bA** on peak $\alpha4\beta2$ currents. With ACh 200 μM, a concentration of 0.3 μM **2bA** produces an increase of 20%, whereas concentrations 1 and 3 μM **2bA** produce an increase of 50% approximately. Increasing the concentration further to 10 μM reduces the current by 15%. This peculiar concentration dependence of the effect of **2bA** on the currents suggests a different action

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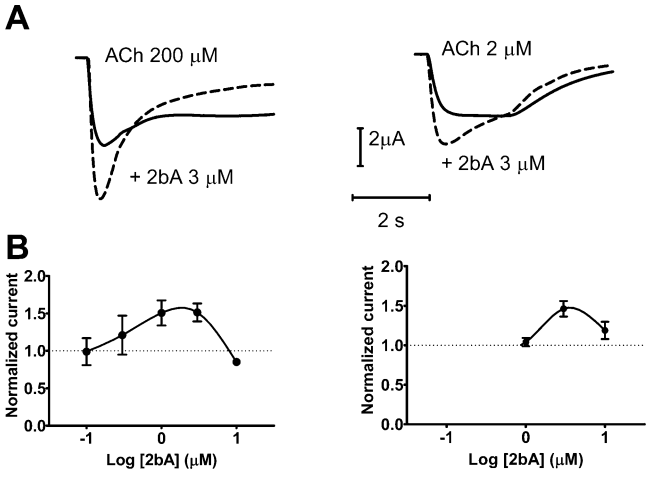

Figure 7. (A) Currents in $\alpha4\beta2$ receptors elicited by ACh 200 μM (left) or ACh 2 μM (right) in the absence (solid line) or the presence (dashed line) of 3 μM **2bA**. (B) Normalized peak current obtained with ACh 200 μM (left) or ACh 2 μM (right), as a function of the **2bA** concentration; the solid line is a spline drawn through the data points and the dotted line is the reference control line.

mechanism that we have tried to understand by the use of a simple kinetic model explained in the next section.

A similar modulatory effect, with potentiation at intermediate concentrations and inhibition at high concentrations, is observed with Zn^{2+} on $\alpha4\beta2$ and $\alpha3\beta4$ receptors expressed in *Xenopus* oocytes, and this effect is strongly dependent on the stoichiometry of the receptors.[7,8] In our case, the potentiating effect of 3 μM **2bA** on the current of $\alpha4\beta2$ receptors in the presence of ACh 200 μM (Figure 7A) is also observed with a much lower concentration of ACh, 2 μM, although in a more limited range of concentrations (Figure 7B), thus suggesting that the effect of Tzs is probably present in both HS and LS $\alpha4\beta2$ receptors.

A Simple Kinetic Model of the Effect of Triazines. The blocking effect of Tzs on NNRs can be modeled by including an additional nonconducting state to the basic kinetic linear model of NNRs. Figure 8C shows a typical linear kinetic model for $\alpha7$ receptors with a resting, unbound state (R), two closed states, bound with one (RA) or two (RA_2) agonist molecules, and an open (OA_2) and desensitized (DA_2) state also with two agonist molecules bound. To account for the inhibitory effect of Tzs, an additional closed state (RB) has been added dependent on the binding of Tzs, with a binding kinetic constant, k_{1b}, proportional to the concentration of the Tzs, and an unbinding kinetic constant, k_{-1b}, independent of this concentration. This simple model predicts well the inhibitory effect of Tzs on $\alpha7$ currents and also its time course as seen in Figure 8A for the specific case of **2dA**, except for the highest concentration of **2dA** (3 μM), where nonzero current value is predicted by the model although the experimental result is a current too small to be measured. The measured peak current as a function of Tz concentration is compared with the prediction of the model in Figure 8C, where the resulting data have been fitted to the Hill equation with parameters (IC_{50} in μM, nH) for the experimental data (0.80 ± 0.06, 1.93 ± 0.25) and for the modeled data (0.77 ± 0.07, 1.57 ± 0.21); both sets of parameters are very similar.

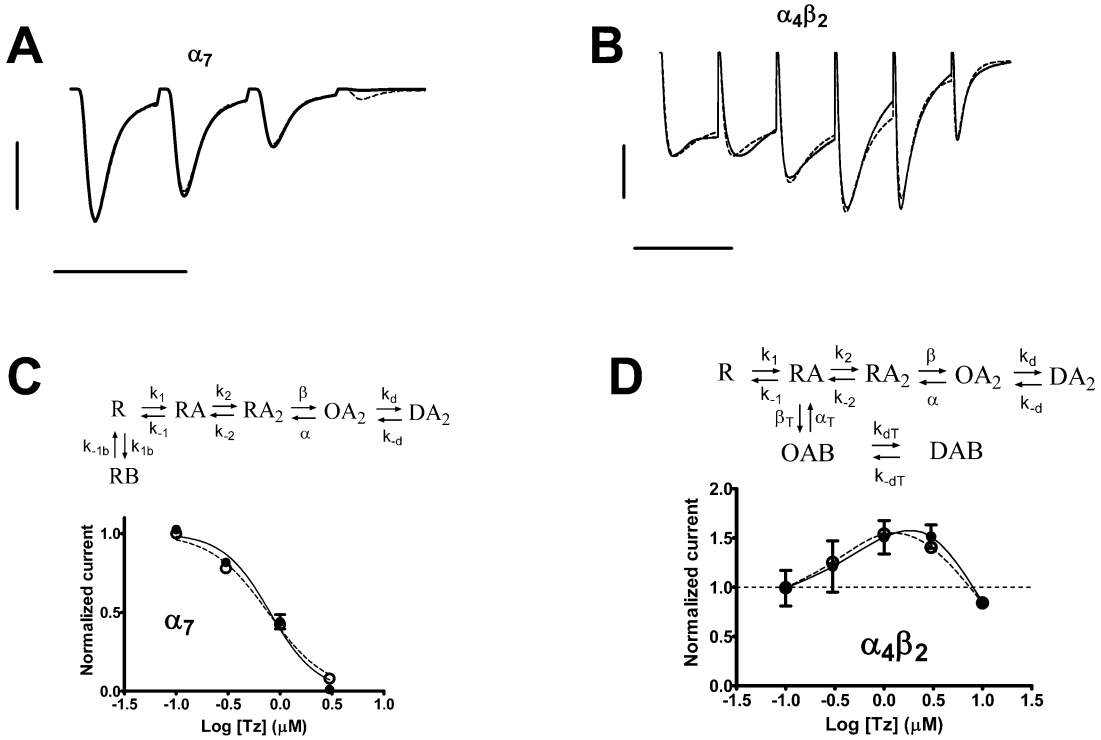

Figure 8. Kinetic model of the effect of Tzs on NNR. (A) Ionic currents elicited by ACh 200 μM in the presence of Tz at 0.1, 0.3, 1, and 3 μM in $\alpha7$ receptors (solid lines). (B) Ionic currents recorded from $\alpha4\beta2$ receptors as in panel A but with an additional concentration of Tz, 10 μM. In both panels, dashed lines correspond to the modeled ionic currents according to the kinetic models described below. (C) Kinetic model used to simulate the effect of Tzs on $\alpha7$ currents, and dose–response curve corresponding to the inhibitory effect of Tz with measured data (solid line and dots) and modeled data (dashed line and open dots). The lines are results of the fit to the Hill equation. (D) Kinetic model used to simulate the effect of Tzs on $\alpha4\beta2$ currents, and dose response with measured data (solid dots) and modeled data (open dots). The lines are splines through the data points.

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Furthermore, the potentiating effect of Tzs on $\alpha4\beta2$ can be modeled by including an additional open state (OAB) with one agonist molecule already bound accessible upon binding by Tzs, and a corresponding desensitized state (DAB), with one molecule of agonist and one molecule of Tz bound, as shown in Figure 8D. Thus, the receptor could open with a single agonist molecule bound in the presence of Tzs, and from this open state, it would transition faster to a desensitized state. The model reproduces well the effect of Tzs on the peak currents and also on the macroscopic kinetics of the current elicited by ACh as shown in Figure 8B,D for the specific case of **2bA**.

The kinetic models presented here are based on a simple sequential linear model for NNRs that is not complete but is sufficient to reproduce some characteristic features of the function of NNRs in the presence of Tzs, that is, the block of $\alpha7$ and the potentiation at intermediate concentrations of $\alpha4\beta2$ receptors. Other kinetic models have been proposed that account for many more properties of nicotinic receptors. They usually include open states with only one agonist molecule bound[9] or even can be open without agonist.[10−12] These models, however, are very complex and have too many kinetic constants to be determined. In any case, we deem that our approach, even being a simple one, provides a satisfactory explanation to the function of NNRs in the presence of the triazine derivatives assayed.

CONCLUSION

In this study, we have investigated the effect of Tzs on the function of NNRs expressed in *Xenopus* oocytes. We have found that Tzs normally inhibit the peak current of NNRs and accelerate the macroscopic kinetics of the currents. However, these effects do not seem to be produced by a simple open channel block mechanism as is the case of the TRP vanilloid 1 receptor, described previously,[5] and the antiepileptic drug lamotrigine on the muscle nicotinic acetylcholine receptor.[13] The blocking effect of Tzs on NNRs is reversible (although the reversibility is slow at high concentrations) and seems to be noncompetitive.

Although some triazines have been found to act as agonists of nicotinic acetylcholine receptors, acting at a site different from those of full agonists,[3] we have not observed any direct activating effect on NNRs of the Tzs studied (data not shown).

On the other hand, the most unexpected effect of Tzs on NNRs is the potentiating effects of **2aD**, **2aF**, **2bA**, and **2gA** on $\alpha4\beta2$ receptors. Other triazines have been described as having only a blocking effect on $\alpha4\beta2$ receptors.[4] By contrast, the aforementioned Tzs have a dual effect, potentiating at intermediate concentrations and blocking at concentrations higher than 10 μM.

A simple kinetic model suggests a possible mechanism of action that would explain this dual effect. For those receptors and Tzs whose combination only produces a reduction in the current, an additional closed state bound with a molecule of Tzs is enough to explain the results. However, the potentiating effect of some Tzs on $\alpha4\beta2$ suggests the existence of a more complex model in which two additional states are necessary; one of them is an open state and the other one a desensitized state. These new states, promoted by the binding of a molecule of Tz, cannot be connected directly to the resting state of the receptor to account for the observation that Tzs by themselves are not able to activate the channel. Thus, in this respect, those Tzs act more like novel allosteric modulators of $\alpha4\beta2$ NNR[14] and could have interest to be further investigated as potential new therapeutic agents.[15]

METHODS

Triazine Derivative Synthesis. The synthesis of title compounds was carried out using the general pathway shown in Figure 1S of the Supporting Information, see also Vidal-Mosquera et al.[5] In this context, the preparation and characterization of the corresponding disubstituted triazine intermediates is also described in the Supporting Information.

Oocyte Expression. All cDNAs were cloned in derivatives of the pSP64T vector[16] containing part of the pBluescript polylinker. Capped mRNA was synthesized in vitro using SP6 RNA polymerase, the mMESSAGE-mMACHINE kit (Applied Biosystems, Madrid, Spain), and the same pSP64T derivatives mentioned above. Defoliculated *Xenopus laevis* oocytes were injected with 5 ng of each subunit cRNA in 50 nL of sterile water. All experiments were performed within 2−3 days after cRNA injection.

Electrophysiological Recordings. Two electrode voltage-clamp electrophysiological recordings in *Xenopus* oocytes were carried out as previously described.[17] The extracellular solution contained (in mM) NaCl 82.5, KCl 2.5, $BaCl_2$ 2.5, $MgCl_2$ 1.0, and HEPES 5 (pH 7.4) The replacement of calcium by barium in this solution diminishes the activation of calcium-activated chloride currents. The velocity of application of agonists through a tube located very close to the oocyte was 18−22 mL/min. The solution exchange rate followed an exponential time course with a time constant of 90 ms.[18]

Unless otherwise specified, triazines were preapplied in the bath for 2 min and then coapplied with the corresponding agonist through a pipet held very close to the oocyte for fast application.

Functional expression of each receptor was estimated as the peak ionic current evoked by 4 s application of 1 mM ACh at −80 mV. All experiments were performed at 22 °C. Current records were measured with Clampfit 10.0 (MDS Analytical Technologies, Sunnyvale, CA, USA). The macroscopic kinetics of the currents was characterized by the 10−90% of the peak rise time, and the 100−50% of the peak decay time. These measurements of rise and decay times are model-independent, avoid the uncertainties inherent to the precise determination of the beginning of the response, and allow characterizing the kinetic behavior of the macroscopic currents even when complex waveforms are present. Because of the interference with electrical noise, accurate measurements of rise and decay times were only possible in those receptors with peak currents greater than 0.1 μA approximately.

Data Analysis. Normalized peak currents were obtained by dividing the maximum value of the current obtained in the presence of the triazine by the maximum value of the current obtained in control conditions. Dose−response curves for the peak current obtained with ACh were fitted to the Hill equation: normalized current = $I_{max}/(1 + (EC_{50}/[ACh])^{nH})$. Dose−response inhibition curves were fitted to the modified Hill equation: normalized current = $1/(1 + ([Tz]/IC_{50})^{nH})$.

Data are expressed as mean ± SEM. Statistical significance was calculated by one-way ANOVA test and, when a significant *F*-value was obtained, by Bonferroni's multiple comparison test. Statistical analyses were performed by GraphPad Prism 5 and IBM SPSS 20 statistics software packages.

Kinetic models were designed with QUB software, and macroscopic rate constants were estimated using the ensemble optimization algorithm.[19]

ASSOCIATED CONTENT

Supporting Information

Synthetic scheme for the preparation of title compounds and the general protocol for the synthesis of Tzs. This material is available free of charge via the Internet at http://pubs.acs.org.

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AUTHOR INFORMATION

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS

ANOVA, analysis of variance; cRNA, capped mRNA; NNR, neuronal nicotinic receptors; Tzs, triazine derivatives

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