Activation, inactivation, and desensitization of acetylcholine receptor channel complex detected by binding of perhydrohistrionicotoxin

(ionic channel/Na+ influx/electric organ/neuromuscular transmission/conformational changes)

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ABSTRACT The effects of receptor activation were studied on the interaction of perhydrohistrionicotoxin (H12-HTX) with the ionic channel of the nicotinic acetylcholine (AcCho) receptor in membranes from the electric organ of Torpedo ocellata and with the endplate region of the soleus muscle of the rat. In Torpedo membranes, the initial rate (i.e., within 30 sec) of [3H]H12-HTX binding to the ionic channel of the AcCho receptor was accelerated 102- to 103-fold in the presence of carbamoylcholine (Carb). H12-HTX also inhibited Carb-activated 22Na+ influx, over 95% inhibition at 10 μM H12-HTX. At this concentration H12-HTX did not inhibit [3H]AcCho binding to the AcCho-receptor sites. There was good correspondence between the degree of acceleration of [3H]H12-HTX binding and the stimulation of 22Na+ influx over a wide range of Carb concentrations (up to 100 μM). Preincubation of Torpedo membranes with Carb decreased the initial rate of [3H]H12-HTX binding, as well as the rate of 22Na+ influx, which may reflect desensitization of the AcCho-receptor. d-Tubocurarine inhibited the agonist-mediated acceleration of [3H]H12-HTX binding and 22Na+ influx in the soleus muscle endplate. H12-HTX inhibited the transient depolarization induced by microiontophoretic application of AcCho; the more receptors activated and channels opened, the stronger was the inhibition by H12-HTX. These findings suggest that H12-HTX binds to closed and open ionic channels, with a preference for the latter conformation. It is also suggested that the conformational changes associated with activation or desensitization of the receptor can be monitored by studying binding of [3H]H12-HTX to the ionic channel sites as well as by the AcCho-receptor-regulated 22Na+ influx.

The acetylcholine (AcCho) receptor of muscle endplate and fish electric organs is an intrinsic protein of the postsynaptic membrane which, upon binding of AcCho, induces transient depolarization of the postsynaptic membrane as a result of activation of ionic channels into conductive or open states (1, 2). AcCho-receptor proteins have been isolated from the electric organs of the electric ray, Torpedo, and the electric eel, Electrophorus, and many of their biochemical properties have been studied (for recent reviews see refs. 3 and 4). While it is agreed that the AcCho receptor and the ionic channel are coupled in the membrane, it is still unclear whether the receptor binding sites and the ionic channel sites are on subunits of the same molecule or are on adjacent interacting molecules (5–7). Biochemical study of this system has been facilitated by the discovery that certain drugs and toxins bind specifically to sites on the AcCho receptor or on its ionic channel. Histrionicotoxin (HTX), an alkaloid isolated from skin secretions of the Colombian frog Dendrobates histrionicus, was found to bind selectively to the ionic channel of the AcCho receptor (8, 9). In electrophysiological studies, HTX was found to react with the ionic channel in both closed (i.e., resting) and open (i.e., active) conformations (2, 10). The action of HTX on the open channel has been shown to be directly dependent upon the degree of agonist-mediated receptor activation (2, 8, 10). Trinitiated perhydrohistrionicotoxin ([3H]H12-HTX), which has similar effects to HTX (7), was used as a specific probe for the in vitro identification of the ionic channel of Torpedo electric organ (6, 7). Binding of [3H]H12-HTX to electric organ membranes was to a finite number of sites that were not the agonist- or antagonist-binding sites on the AcCho receptor, and its binding was inhibited selectively by drugs and toxins that affected the time course of endplate currents, an indication of blockade of the ionic channel (2, 5, 6).

Receptor-regulated ion flux measurements in microsacs prepared from Electrophorus and Torpedo electric organs can be used to monitor the presence of a functional complex of AcCho-receptor and its ionic channel and to identify the components required for their function. An early study of AcCho receptor-mediated ionic fluxes in vitro established that electric organ membranes formed sealed microsacs and AcCho-receptor activation caused increased efflux of entrapped 22Na+ (11).

The objective of the present investigation is to elucidate the influence of AcCho-receptor activation on the reaction of H12-HTX with the ionic channel sites in microsacs from the Torpedo electric organ and in endplates of rat soleus muscle. In conjunction with a rapid influx assay, a filter method was used to measure the kinetics of binding of [3H]H12-HTX. The biophysical and biochemical data show that receptor activation by agonists significantly increases the reaction of H12-HTX with the ionic channel sites. Binding of [3H]H12-HTX to ionic channel sites can be utilized to detect changes in conformations of the AcCho receptor–ionic channel complex and interactions between the receptor and channel sites. The influence of AcCho-receptor activation on the binding of ligands to the ionic channel sites was evident in studies of the kinetics of both [3H]H12-HTX binding and the receptor-regulated 22Na+ influx.

MATERIALS AND METHODS

Membrane Preparation. The electric organ of Torpedo ocellata (collected from the Mediterranean and stored at −90 °C for up to 6 months) was homogenized in an equal volume of an ice-cold solution of 10 mM Tris-HCl, pH 7.4, containing 1 mM Na2EDTA, 0.02% NaN3, 0.1 mM diisopropylfluorophosphate (iPrF-F), and 0.1 μM phenylmethylsulfonyl fluoride. The pellets of a 10-min centrifugation at 5000 × g were resuspended in the same buffer and respun at 5000 × g

Abbreviations: AcCho, acetylcholine; Carb, carbamoylcholine; HTX, histrionicotoxin; H12-HTX, perhydrohistrionicotoxin; dTC, d-tubocurarine; iPr2F-F, diisopropylfluorophosphate.

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for 10 min; then the supernatants were combined and centrifuged at 30,000 × g for 60 min. The pellets were resuspended in 10 mM Tris-HCl, pH 7.4/0.02% NaN₃/0.1 mM iPr₂P-F, at a protein concentration of 1–2 mg/ml, as determined by the method of Lowry et al. (12). The number of AcCho-receptor sites ranged from 0.5 to 1 nmol/mg of protein.

**Binding Measurements.** [³H]H₁₂-HTX (specific activity 21 Ci/mmol; 1 Ci = 3.7 × 10¹² becquerels) was obtained by tritiation of isodihydriostionictoxin, and its activity was tested on frog sartorius muscle as described (5). Binding of [³H]-H₁₂-HTX was determined by a filtration assay in which a membrane preparation (=50 μg of protein) was incubated at 22°C in 50 mM Tris-HCl, pH 7.4, and [³H]-H₁₂-HTX at the required concentration in a final volume of 1 ml. Receptor drugs were added either prior to or simultaneously with [³H]-H₁₂-HTX, and the incubation period varied depending on the purpose of the experiment. The mixture was filtered on Whatman GF/B glass-fiber filters, and the filters were rinsed once with 7 ml of Tris buffer. Each filter was placed in a 10-ml Filmware bag (Nalge/Syrbron), 5 ml of toluene-based scintillation solution was added, and after 10 hr the radioactivity was measured in a liquid scintillation spectrometer. Specific binding to the ion channel sites was obtained after subtraction of nonspecific binding from the total binding. Because we had shown that amantadine (13) and quinacrine (14) interacted with the ion channel and inhibited its binding of [³H]-H₁₂-HTX competitively, [³H]-H₁₂-HTX binding that was not inhibited by 0.1 mM quinacrine or 5 mM amantadine was considered to be nonspecific; it amounted to about 13% of the total binding at equilibrium. Binding of [³H]AcCho (49.5 mCi/mmol, New England Nuclear) was measured by equilibrium dialysis for 4 hr at 23°C in Krebs original Ringer’s phosphate solution containing 100 μM iPr₂P-F as described (7). Further details of the filter assay, including saturability of [³H]-H₁₂-HTX binding, pharmacologic specificity, and its linear relationship to tissue concentration as well as additional kinetic analyses of receptor-ionic-channel interactions will be published elsewhere.

**Flux Measurements.** Torpedo membranes used in flux measurements were prepared in the manner described above except that the buffer did not contain EDTA or iPr₂P-F. Sequestered ²²Na⁺ was separated from free ²²Na⁺ by the method of Epstein and Racker (15) with some modifications. Dowex 50W-X8 resin, 100–200 mesh, was converted to the Tris form by equilibration in 10 mM Tris buffer, pH 7.2. One milliliter of the heavy slurry was packed into a pasteur pipette loosely plugged with glass wool and kept under buffer at 4°C till use. For influx measurement 200 μl of Torpedo membranes (10–15 mg of protein per ml) was added to a test tube containing 0.1 μCi of ²²Na⁺ in 200 μl of Tris buffer plus 5 μl of carbamoylcholine (Carb) solution. The contents of the tube were mixed and a 300-μl aliquot was transferred after 30 sec to the Dowex column. Slight air pressure helped the sample to sink in the resin immediately. Then the sample was washed through the column with 1 ml of buffer under mild air pressure. The eluate was collected and counted in a Packard gamma counter. Carb-stimulated influx was calculated as the difference in ²²Na⁺ content of Torpedo microsacs in the presence and absence of Carb. In the presence of saturating concentrations of Carb the amount of ²²Na⁺ influx was 2–3 times greater than the influx in the absence of Carb.

**Measurements of AcCho Sensitivity.** The soleus muscles of rats (Wistar) were dissected in a solution containing the following in mM concentrations: NaCl, 135.0; KCl, 5.0; CaCl₂, 2.0; MgCl₂, 1.0; NaHCO₃, 15.0; NaH₂PO₄, 1.0; and glucose, 11.0. The same buffer was used during the experiment and was bubbled with 95% O₂/5% CO₂ giving a pH of 7.1–7.3. The muscles were treated with 500 mM glycerol to inhibit contractions, and measurements were made as described (9). Microiontophoretic application of AcCho was from high-resistance pipettes (250–450 MΩ) filled with 2.5 M AcCho. The duration of the cathodal current pulse varied from 0.05 to 5 msec, and this current pulse was superimposed on a low constant anodal braking current. When an increase in charge applied to the pipettes was required, precautions were taken to avoid capacitance leakage.

**RESULTS**

**Effects of Carb on ²²Na⁺ Influx and Rate of [³H]H₁₂-HTX Binding.** The binding of [³H]H₁₂-HTX (2 nM) to Torpedo membranes in the absence of receptor agonists was slow (Fig. 1). At room temperature equilibrium was reached after 60 min of exposure, whereas at 4°C it was not reached even after 7 hr. Simultaneous addition of Carb, a receptor agonist, with [³H]-H₁₂-HTX accelerated the rate of binding of the labeled toxin in a dose-dependent manner (Fig. 1). Maximum acceleration was obtained with 32 μM Carb or AcCho, which caused two to three orders of magnitude increase in the amount bound after 30-sec exposure to [³H]H₁₂-HTX (i.e., the initial rate of binding). The half-time of [³H]-H₁₂-HTX binding to reach equilibrium in the presence of such high concentrations of agonists was reduced from 20 min to <1 min. The level of binding of 2 nM [³H]-H₁₂-HTX at equilibrium was about 20% lower in the absence than in the presence of Carb (10 μM). The apparent dissociation constant (Kₐ) of [³H]-H₁₂-HTX binding, measured by inhibition of [³H]-H₁₂-HTX binding with unlabeled H₁₂-HTX, was 110 ± 4 nM in the absence and 82 ± 4 nM in the presence of 1 μM Carb. The dissociation rate constant (kᵣ⁻¹) measured by displacing [³H]-H₁₂-HTX with mM quinacrine after equilibration of membranes and [³H]-H₁₂-HTX for 30 min in the absence of receptor agonists was 4 × 10⁻³ sec⁻¹, and this rate was not changed significantly in the presence of Carb.

Because the initial rate of [³H]-H₁₂-HTX binding was greatly stimulated by receptor activation, while the affinity for [³H]-H₁₂-HTX measured at equilibrium was increased only 2-fold, it is apparent that the initial rate of [³H]-H₁₂-HTX binding is influenced by factors that do not contribute greatly to the final equilibrium position. The calculated total number of binding sites for [³H]-H₁₂-HTX was 0.4 ± 0.2 (range 0.3 to 0.55) nmol/mg of protein in the absence and 0.5 ± 0.1 (range 0.4 to 0.6) nmol/mg of protein in the presence of 1 μM Carb, which equals 0.7 of the AcCho or α-bungarotoxin binding sites in the tissue. These concentrations are similar to the values determined by the centrifugal assay (7). Apparently, the initial rate of [³H]-H₁₂-HTX binding is influenced by factors that do not contribute greatly to the final equilibrium position.

The uptake of ²²Na⁺ into Torpedo microsacs was also stim-

![Fig. 1. Effects of different concentrations of Carb on ²²Na⁺ uptake into Torpedo microsacs.](image)
ulated by Carb (Fig. 1). Pretreatment of *Torpedo* membranes with α-bungarotoxin (1 μM) for 30 min resulted in complete inhibition of the Carb-stimulated 22Na⁺ uptake (data not shown). Plotting the fractional response of 22Na⁺ influx and initial rate of [3H]H₁₂-HTX binding at various Carb concentrations gave a linear relationship between 1 and 320 μM (correlation coefficient = 0.98). However, higher concentrations of Carb reduced both to slightly different extents.

**Effect of Receptor Activation on the Action of H₁₂-HTX on the Ionic Channel.** The effect of H₁₂-HTX (35 μM) was studied on the postsynaptic membrane depolarizations caused by the microiontophoresis of AcCho in five consecutive pulses inside the endplate of rat soleus muscle. The fifth membrane depolarization was always more depressed than the second and first membrane depolarizations (Table 1). The second depolarization was depressed to 79% of the first depolarization when the duration of the AcCho pulse was 0.05 msec, and depression increased when longer pulses were used, reaching 19% of the first depolarization after a 5-msec pulse. Thus, the longer the pulse duration, the more the AcCho released and the stronger the depression of AcCho sensitivity (Table 1). This relationship was evident whether the second or fifth depolarization was compared to the first. It suggested that the more channels were activated by the action of AcCho the stronger was the inhibiting effect of H₁₂-HTX, and that therefore H₁₂-HTX interacted with the activated ionic channel, in addition to its previously described interaction with the closed ionic channel measured in denervated muscle in the absence of AcCho (10).

**Effect of Desensitization on 22Na⁺ Influx and the Rate of [3H]H₁₂-HTX Binding.** If the increase in initial rate of [3H]-H₁₂-HTX binding is due to the toxin's increased binding to an activated channel conformation, it would be expected that desensitizing concentrations of Carb reduce the rate of [3H]-H₁₂-HTX binding because of an increase in the population of inactive receptor-channel complexes. As shown in Fig. 1, when Carb and [3H]H₁₂-HTX were added to the tissue simultaneously, Carb at concentrations in excess of 100 μM decreased the initial rate of [3H]H₁₂-HTX binding in a dose-dependent manner. When Carb and 22Na⁺ were added simultaneously to the microsacs in measurements of 22Na⁺ influx, indications of desensitization appeared only at Carb concentrations above 100 μM (Fig. 1). It is interesting to note that the decrease in 22Na⁺ uptake by high concentrations is less than the decrease in the rate of [3H]H₁₂-HTX binding (Fig. 1). However, when the microsacs were preincubated with Carb for a period prior to the influx measurements, a reduction in flux activity was observed with Carb concentrations as low as 1 μM (Fig. 2A). The reduction of total influx as well as the rate of its onset were positively correlated with the concentration of the agonist. Preexposure of *Torpedo* membranes to Carb also reduced the initial rate of [3H]H₁₂-HTX binding in a time-dependent manner (Fig. 2B), and was evident even with as little as 1 μM Carb.

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![Fig. 2](image2.png)

**Fig. 2.** The effects of preincubation of *Torpedo* microsacs with Carb for various times on the degree of 22Na⁺ uptake (A) and on the initial rate of [3H]H₁₂-HTX binding (B). (A) Before exposure to 22Na⁺ and 100 μM Carb, each microsac sample was incubated for the indicated time with one of the following Carb concentrations in μM: 0, 0.1; A, 1; B, 2; O, 5; and X, 10; influx was measured after 30 sec. Control influx was measured with no preexposure to Carb. (B) Binding of 2 nM [3H]H₁₂-HTX was either in the absence (O) or the presence of 10 μM Carb, in which case preincubation with 10 μM Carb was for one of the following times in min: 0; A, 1; B, 3; O, 15. Each symbol represents the mean of three experiments; the SD of each was less than 10%.

**Effect of d-Tubocurarine (dTC) on 22Na⁺ Influx and Rate of [3H]H₁₂-HTX Binding.** dTC inhibited the Carb-stimulated 22Na⁺ influx in a dose-dependent manner with a Ki of 0.24 ± 0.04 μM. Concentrations of dTC ranging from 0.1 to 100 μM did not affect 22Na⁺ influx in the absence of Carb. When Carb-stimulated 22Na⁺ flux was measured in presence of a constant concentration of dTC, there was a shift in the dose-response relationship to the right (Fig. 3). This result confirms the action of dTC at these concentrations as a competitive antagonist of receptor function. The rate of [3H]H₁₂-HTX binding in presence of 1 μM Carb was also reduced by dTC in a dose-dependent manner (Fig. 4). However, dTC alone at 10 μM
increased \([3H]H_{12}\)-HTX binding in the absence of Carb (Fig. 4), and this increase was dose dependent (data not shown). Fifty percent inhibition by dTC of the Carb-stimulated \([3H]H_{12}\)-HTX binding at 30 sec was produced by 0.6 \(\mu M\) dTC after corrections for the stimulation caused by dTC at various concentrations.

**Effect of \(H_{12}\)-HTX on \(^{22}Na^+\) Influx and \([3H]AcCho Binding.** To test the effect of \(H_{12}\)-HTX on AcCho-receptor-activated \(^{22}Na^+\) flux, the *Torpedo* microsacs were first incubated with 1 \(\mu M\) Carb and the desired concentration of the toxin for 2 min. Then the microsacs were added to a solution containing \(^{22}Na^+\) and 100 \(\mu M\) Carb and the influx was measured after 30 sec. \(H_{12}\)-HTX inhibited receptor-activated ionic fluxes in a dose-dependent manner (Fig. 5). At the concentrations used, \(H_{12}\)-HTX did not reduce the binding of \([3H]AcCho to Torpedo membranes. Thus the inhibition by \(H_{12}\)-HTX of \(Na^+\) flux activation occurred as a result of the direct action of the toxin on the channel sites rather than through an effect on the receptor sites.

**DISCUSSION**

It is evident that \([3H]H_{12}\)-HTX can be utilized as a biochemical probe to monitor states of activation, inactivation, and desensitization of the AcCho receptor–channel complex that are affected by binding of ligands to the AcCho-receptor sites. There is good correlation between Carb-stimulated \(^{22}Na^+\) influx into *Torpedo* microsacs and the initial rate of the binding of \([3H]H_{12}\)-HTX in several parameters. Carb stimulates uptake of \(^{22}Na^+\) and accelerates \([3H]H_{12}\)-HTX binding at similar concentrations. Both events, however, seem to be inhibited with desensitizing concentrations of Carb (Fig. 2) or by the presence of dTC (Figs. 3 and 4). The correspondence between the effects of the same Carb concentrations in stimulating \(^{22}Na^+\) influx and initial rate of \([3H]H_{12}\)-HTX binding, as well as the inhibition of AcCho-receptor-activated \(^{22}Na^+\) flux by inhibitors of both the AcCho-receptor sites and the ionic channel sites (Fig. 5; refs. 7, 13, 14), suggests that the \(^{22}Na^+\) influx measurements may reflect the function of the AcCho-receptor and its ionic channel. The influx of \(^{22}Na^+\) into microsacs, which can be measured in seconds, is still too slow to detect various states of the AcCho-receptor ionic channel complex. However, the method is fast enough to detect initial stages of desensitization of the AcCho receptor caused by preexposure to agonists (Fig. 2). It is important to note that although the action of \(H_{12}\)-HTX on the endplate currents is voltage and time dependent (2, 10), it is not known if there is a potential difference across the microsac membranes used in the binding and \(^{22}Na^+\) influx studies. If the membrane potential is indeed near zero, then the stimulated rate of \([3H]H_{12}\)-HTX binding presently observed is much smaller than what would occur in vivo.

Earlier biochemical evidence for allosteric interactions between ionic channel sites and AcCho-receptor sites was based on the finding of increased \([3H]AcCho binding (16, 17), or changes in the fluorescence of ligands bound (18, 19) to the receptor sites, as a result of local anesthetic or HTX interaction with ionic channel sites. Carb stimulates \(^{22}Na^+\) influx into microsacs by reacting with the AcCho-receptor and causing activation of the ionic channel (Figs. 1 and 4). The agent also increases the initial rate of binding of \([3H]H_{12}\)-HTX to ionic channel sites (Figs. 1 and 2). The good correlation between the \(^{22}Na^+\) influx and binding data confirms the electrophysiological findings (Table 1) that activation of the AcCho receptor potentiates the inhibitory effect of \(H_{12}\)-HTX, as was found with HTX (2, 10). These and previous studies (2, 9, 10) on skeletal muscle endplates suggest that \([3H]H_{12}\)-HTX reacts with the open and closed conformations of the ionic channel of the AcCho receptor, and the accessibility of these sites in the open channel conformation is affected by the voltage across the membrane and the time of exposure to the voltage. These findings suggest extreme diffusional barriers between \([3H]H_{12}\)-HTX and its sites of action on the ionic channel, barriers that are substantially lowered when the channel is activated. The finding that dTC increases the initial rate of \([3H]H_{12}\)-HTX binding without causing any specific \(^{22}Na^+\) influx suggests that there may be an inactive conformation of the ionic channel in which \([3H]H_{12}\)-HTX binding sites are more accessible. Hence, this effect may be explained by assuming the presence of more than one inactive channel conformation in equilibrium, in which dTC binding to the AcCho receptor favors the conformation that has more accessible sites for \([3H]H_{12}\)-HTX. Alternatively, it may be that dTC binding to the receptor–channel complex slows the dissociation of \([3H]H_{12}\)-HTX, resulting in higher apparent binding. However, we have no evidence to back either possibility, and this effect of dTC remains unex-
explained. The enhancement of [3H]H12-HTX binding is not a property of all receptor antagonists, because we found that whereas gallamine and benzquinonium stimulated the initial rate of [3H]H12-HTX binding, α-bungarotoxin did not (unpublished results). It should be noted that dTC not only inhibits nicotinic receptor sites, but in higher concentrations it also shortens the time course of endplate currents (20, 21), a property common to several toxins and drugs that interact with the ionic channel (2). Thus, dTC inhibition of the AcCho receptor-regulated 22Na+ influx may result from one or both of these actions.

It has been suggested that agonist association with, and dissociation from, the AcCho receptor is rapid, whereas conversion of the agonist–receptor complex to the desensitized state is much slower (22). Exposure of electric organ membranes to agonists such as Carb has been shown to cause a slow conversion of the receptor from a low- to a high-affinity state for agonist binding, which was proposed to be to a desensitized state. This conversion was revealed by toxin competition (23) as well as by electron spin resonance spectrometry (24). Local anesthetics were also found to convert the AcCho receptor to the high-affinity state for agonist binding, which led to the suggestion that perturbation of the hydrophobic environment of the membrane may cause the conversion of the receptor (25). Similarly, it was suggested that H12-HTX caused stabilization of the AcCho receptor of cultured chick muscle cells in the high-affinity desensitized state (26). The inhibition of stimulated [3H]H12-HTX binding (Fig. 2B) and the 22Na+ uptake (Fig. 2A) resulting from preincubation with Carb, which is dependent upon Carb concentration and the time of exposure, may reflect a conformational change of the receptor into a desensitized state. The time-dependent change in receptor-channel conformation is evident from the observation that preincubation in as little as 1 μM Carb inhibits the stimulated 22Na+ influx and [3H]H12-HTX binding (Fig. 2), whereas no reduction is observed in the 22Na+ flux or toxin binding (Fig. 1) when Carb is present simultaneously with the 22Na+ or toxin at concentrations up to 32 μM. It is suggested that the flux or binding observed is a summation of the contributions of active, inactive, and desensitized conformations of the receptor–channel complex, and that the binding of [3H]H12-HTX to the ionic channel is lower when receptors are in a desensitized state. Thus this binding may detect the conformational change of the receptor into a desensitized state (15, 27, 28).

In comparing the present flux data with previous reports, we find that in other studies higher concentrations of Carb were required to detect desensitization—e.g., 100 μM (15)—compared to as low as 1 μM in our case. The half-time for the initial detectable phase of desensitization with 100 μM Carb was calculated in one case to be ≈3.3 min (28), whereas in our study it was too fast to measure, clearly shorter than 20 sec. This is closer to the estimate of 1.9 sec for receptor desensitization detected by electron spin resonance studies of the binding of the nitroxide analog of decamethonium to Torpedo receptors (25). The half-life for desensitization observed in 22Na+ influx studies was calculated to be as low as 3 sec after exposure to 200 μM Carb for only 1 sec (15). These differences may be due to the fact that preloading with 22Na+ for 6–24 hr (27, 28) is necessary in the 22Na+ influx studies, but not in the influx studies (15) used in the present study. Also, the temperature at which fluxes were monitored varied from 4°C (15, 27, 29) to 22°C (the present study). The isomerization of the AcCho receptor to a desensitized state and the dose dependence of its onset are consonant with electrophysiologically detected AcCho-receptor desensitization.

In summary, both biochemical and biophysical methods show that activation of the AcCho receptor by agonists increases the rate of H12-HTX binding to the ionic channel sites. It is suggested that the rate of [3H]H12-HTX binding to the ionic channel of the AcCho-receptor of Torpedo membranes can be utilized to detect conformational changes in the receptor–channel complex caused by activation or desensitization of the receptor.