BLOCKING KINETICS AT EXCITATORY ACETYLCHOLINE RESPONSES ON *APLYSIA* NEURONS

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Over the last decade, studies of the action of cholinolytic compounds in vertebrate neuromuscular junction and *Aplysia* neurons have shown that most of the compounds long regarded as acetylcholine (ACh)-receptor antagonists do not act primarily at the receptor, as had been supposed, but rather they block the open state of the ACh-activated ionic channel (Adams, 1981; Steinbach, 1980). We have employed the technique of relaxation analysis to examine the effects of several novel ACh antagonists on excitatory ACh responses on *Aplysia* neurons. The effects of strychnine, the calcium antagonist D-600, and the tricyclic antidepressant, desipramine, all compounds with reported cholinolytic actions in other preparations, have been compared with those of the ACh ion-channel–blocking drugs, hexamethonium and atropine. The results of these experiments indicate that strychnine and desipramine act by a mechanism likely to be receptor blockade, whereas D-600 blocks the open ionic channel.

METHODS AND RESULTS

Experiments were performed on *Aplysia* abdominal RB neurons voltage-clamped with two microelectrodes. Agonists were applied by ionophoresis and antagonists were bath-perfused. The effects of drugs on the steady-state (I_{ss}) and voltage-jump ACh or carbachol-induced current relaxations were studied over a range of membrane potentials (−50 to −130 mV) at equilibrium concentrations of both agonist and antagonist. D-600, hexamethonium, and atropine reduced I_{ss} in a voltage-dependent manner, the degree of blockade increasing with hyperpolarization (Fig. 1). In contrast, the reduction of I_{ss} by strychnine and desipramine was not voltage-dependent, the degree of blockade being equal at all membrane potentials for a given concentration of the drug (5–20 μM). The time constant of voltage-jump–induced current relaxations (τ_f) in the presence of ACh or carbachol were reduced in a voltage-dependent manner by D-600, hexamethonium and atropine, the degree of reduction of τ_f increasing with membrane potential (Fig. 2). The reduction of I_{ss} by strychnine and desipramine, however, was not accompanied by any change in τ_f over the range of membrane potentials studied. In normal seawater the ACh-induced inward current exibited a single exponential relaxation to a stable plateau (I_{ss}) following voltage command steps from −50 to between −70 and −130 mV. In the presence of the voltage-dependent antagonists D-600, hexamethonium and atropine, a slow, inverse relaxation followed the accelerated inward relaxation (Fig. 2 *B*). The rate of this inverse relaxation (τ_s) was accelerated by increasing concentrations of either agonist or antagonist, and by increasing

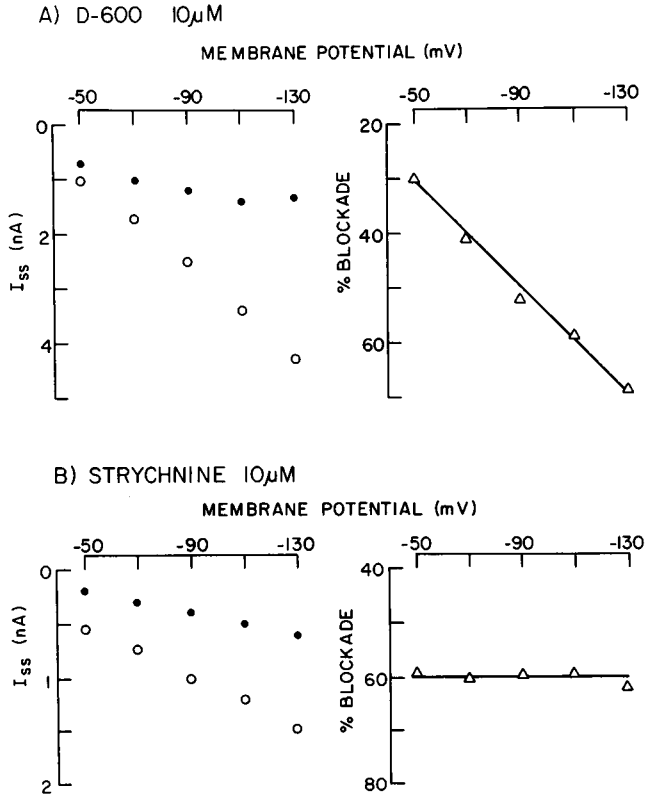

FIGURE 1 Effects of antagonists on the steady-state ACh current-voltage relationship. Left-hand graphs show ACh *I-V* relations in the absence (open circles) and presence (filled circles) of antagonist. Right-hand graphs show degree of antagonism as a percent of control at each membrane potential. (*A*) D-600 10 μM. (*B*) Strychnine 10 μM. Data from two separate cells clamped at −50 mV and stepped to between −70 and −130 mV for 4 s at each potential.

BIOPHYS. J. © Biophysical Society · 0006–3495/84/01/24/02 $1.00
Volume 45 January 1984 24–25

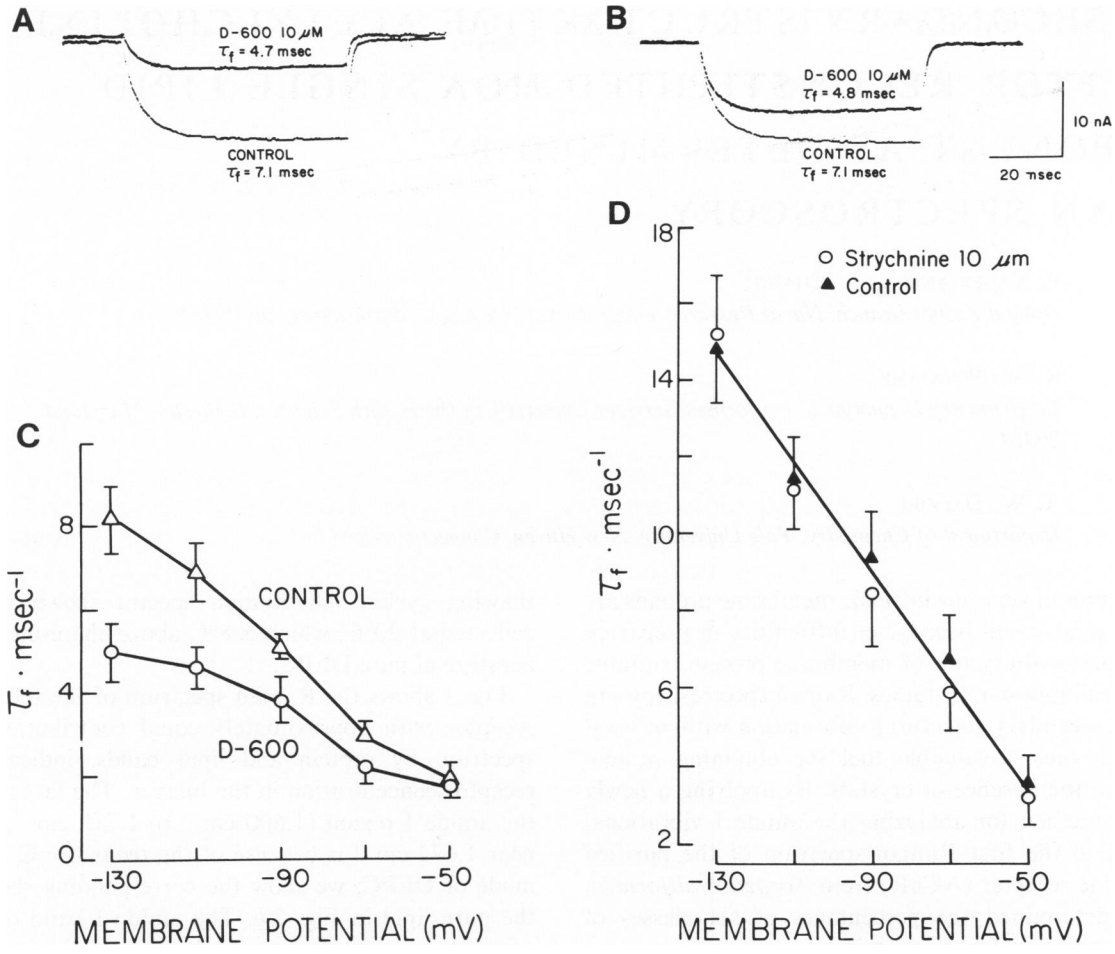

FIGURE 2 Effects of antagonists on mean channel lifetime estimated as the time constant of current relaxations (τ_f) following voltage command steps. (*A*, *B*) Current relaxations obtained by subtraction of current recorded before and during plateau phase of a response to ACh. Cell was clamped at −50 mV and stepped to −130 mV for 60 ms. (*A*) reduction of τ_f by D-600 at same ACh dose as control relaxation. (*B*) same cell as in *A*, ACh dose increased in the presence of D-600 to obtain the same plateau response at the holding potential. Note the appearance of a slow, inverse relaxation in the presence of D-600. (*C*, *D*) Relationship between τ_f and membrane potential for 10 μM D-600 (*C*, $n = 5$) and 10 μM strychnine (*D*, $n = 7$). Points represent mean ± SEM. Temperature = 15°C (*A*, *B*, *C*), 12°C (*D*).

membrane potential. No inverse relaxation was observed in the presence of either strychnine or desipramine.

Two modes of action of cholinolytic agents are apparent in these observations. Strychnine and desipramine block the response in a time- and voltage-independent manner, with no effect on channel lifetime, suggesting an interaction with the receptor moiety. D-600, hexamethonium, and atropine block this response in a time- and voltage-dependent manner. Two kinetic components of the antagonism produced by these drugs can be observed: a fast kinetic component associated with the voltage-dependent reduction of both steady-state currents and channel lifetime, and a slow component dependent on the fraction of open channels and antagonist concentration. These two kinetic components may represent two different sites of interaction by these compounds with the open state of the receptor-ionophore complex.

Received for publication 21 April 1983.

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