amount of $preD_3$ that is formed in the various layers will be dependent on the amount of ultraviolet radiation reaching each layer. Once formed in the skin, $preD_3$ is isomerized to $D_3$ by a nonphotochemical rearrangement of the $preD_3$ triene system at a rate dictated by the temperature of the skin. At 37° ± 1°C, this thermal isomerization reaction appears to be more rapid in vivo than in vitro (Fig. 1B). This difference seems most likely to be due to the specific translocation, by the vitamin D-binding protein, of the thermal product, $D_3$, from the skin into the circulation (in the capillary bed) beneath the dermoepidermal junction. The net effect of this highly specific translocation would be to remove the product $D_3$ (as it is being formed) from the reaction, thereby promoting the conversion of $preD_3$ to $D_3$. Therefore a true equilibrium is never established (as occurs in vitro) and essentially 100 percent of $preD_3$ converts to $D_3$. It is unlikely that the differences between the thermal isomerization of $preD_3$ to $D_3$ in vitro and in vivo are due to a cutaneous catalytic process (such as an enzymatic, lipid-lipid, or lipid-protein interaction), since thermal equilibration time courses for incubations of plasma and skin homogenates with $preD_3$ at 37° ± 1°C were identical to those with $preD_3$ in methanol at 37° ± 1°C. Furthermore, there was no evidence that $preD_3$ was metabolized more rapidly than $D_3$ in the skin, nor was there any difference in the disappearance of radiolabeled $preD_3$ and $D_3$ given intravenously, a finding which suggests that there is no significant difference in the metabolic clearance rates of these two secosterols.

The physiological advantage of photosynthesizing $preD_3$ in the skin during exposure to the sun is illustrated in Fig. 2. During exposure to sunlight, the ultraviolet B portion of the solar spectrum produces the photochemical conversion of cutaneous 7-DHC to $preD_3$. Immediately upon its formation, $preD_3$ begins to isomerize by a temperature-dependent process to $D_3$. This process permits the skin to continually synthesize (from its previtamin) and release $D_3$ into the circulation for up to 3 days after a single exposure to sunlight. In warm-blooded animals, this equilibrium reaction remains relatively unchanged inasmuch as the body temperature is closely regulated; in cold-blooded species, the body temperature fluctuates greatly and therefore this equilibrium reaction would be highly variable. Once $D_3$ is formed, the vitamin D-binding protein translocates it preferentially into the circulation and ensures the efficient conversion of the small quantities of $preD_3$ to $D_3$ by shifting the $preD_3 \rightleftharpoons D_3$ reaction to the right at the time when equilibrium is being approached. Thus, the skin serves as (i) the site for the synthesis of 7-DHC, (ii) a reservoir for the storage of the primary photoproduct, $preD_3$, and (iii) the organ where the slow thermal conversion of $preD_3$ to $D_3$ occurs.

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10 March 1980; revised 27 May 1980

## Asymmetry of the Acetylcholine Channel Revealed by Quaternary Anesthetics

Abstract. *Tissue-cultured rat myoballs were examined electrophysiologically with a suction pipette, which was used for voltage clamping and internal perfusion. The lidocaine derivative QX-314 caused a time- and membrane potential–dependent block of acetylcholine-induced current only when applied from the extracellular membrane surface. The same compound caused a use-dependent block of the sodium channel only from the intracellular membrane surface. These experiments demonstrate a fundamental asymmetry of the acetylcholine receptor-channel complex.*

Pharmacological agents are frequently used to examine the inside-outside symmetry of membrane-bound proteins. The strategy is to examine the effects of a drug when applied to either the intracellular or extracellular membrane surface. Recent electrophysiological data suggest that, in the acetylcholine (ACh) channel, ion permeation occurs through a symmetrically shaped pore (*1*). We tested this postulate by studying the action of ACh channel blockers on both membrane surfaces of tissue-cultured rat muscle cells. We report an asymmetrical action of the lidocaine derivatives QX-314 (quaternary *N*-ethyl) and QX-222 (quaternary *N*-methyl) on the ACh-induced current of these cells.

Myoballs were grown from tissue-cultured muscle of neonatal rat thighs (*2*).

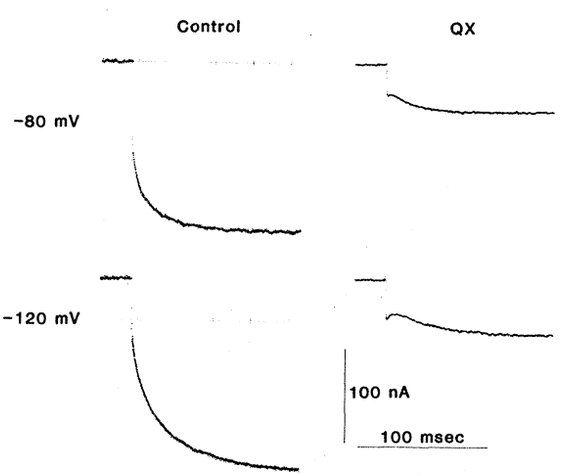


Fig. 1. Effect of external QX-314 (0.1 m*M*). The control traces (left) show the ACh current relaxations for voltage jumps to − 80 mV and − 120 mV from the holding potential of − 40 mV. In each case the current was fitted by a single exponential [$\tau(-80) = 15.3$ msec; $\tau(-120) = 22.9$ msec]. Five minutes after 0.1 m*M* QX-314 was added to the bath, the traces on the right were recorded. The iontophoretic current was approximately the same as in the control case. Derivative QX-314 caused a time- and voltage-dependent reduction of the ACh current (temperature, 18°C).


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The myoballs were internally perfused by a glass suction pipette, which also contained the current electrode. Transmembrane potential was recorded by a separate microelectrode (3*M* KCl, 5 to 25 megohms). The details of the tissue culture procedure, perfusion, and voltage-clamp circuitry were described previously (*2*).

In all experiments, the inside of the pipette was continuously perfused (~ 1 ml/min) with sodium aspartate solution while the cell was bathed in sodium aspartate–Ringer solution (*3*). The cell was voltage-clamped to a holding potential of − 40 mV, and ACh was applied iontophoretically near the cell surface by means of a microelectrode. Voltage jumps were applied both before and during ACh application, and control traces were subtracted digitally from ACh traces to remove capacitative and leakage currents. The iontophoretic current was maintained at a low level to ensure that the rate constant for channel closing was always much greater than that for opening (*4*). Thus the voltage jump time constants were not affected by iontophoretic ACh dosage at any membrane potential. In some experiments, the cationic local anesthetics QX-314 and QX-222 were added to the internal or external solutions.

Voltage jumps of − 40 and − 80 mV produced an exponential relaxation of the ACh-dependent current (Fig. 1). The voltage dependence of the time constant $\tau$ (exponential change per ~ 100 mV) is similar to that reported in other ACh channels, where $\tau$ was determined by noise analysis, voltage jumps, or decay rate of synaptically induced end-plate currents (*5*). The addition of 0.1 m*M* QX-314 to the external solution produced a time- and voltage-dependent alteration of the voltage jump current (Fig. 1). This effect has been described by a sequential blocking model in which the cationic anesthetic enters open ACh channels and blocks in a voltage-dependent manner (*6, 7*). In the presence of QX-314, the current in response to a hyperpolarizing voltage step has a fast (conductance-decreasing) and a slow (conductance-increasing) component. According to the sequential model, the fast component is due to a decrease of current as voltage drives the blocking molecule into open channels. The slow component represents the relaxation of the current to the equilibrium state at the hyperpolarized potential. This state is determined by the normal voltage sensitivity of the ACh channel and by the depth of the anesthetic block (*6, 7*).

The dissociation constant for the binding of extracellular QX-314 to the open channel is voltage-dependent. The voltage dependence can be derived from a comparison of the control time constants during voltage jumps with the slow time constants in the presence of QX-314 (*8*). From this information it is possible to calculate an apparent electrical location of the binding site. For the experiment represented in Fig. 1, the binding site was located about 72 percent of the way through the electrical field from the extracellular surface. This is similar to previously reported electrical distances for binding of anesthetic to the ACh channel (*6, 7*).

In another cell, QX-314 was added to the internal solution at a higher concentration (1 m*M*). Even after perfusion for more than 90 minutes the voltage jump current remained exponential (Fig. 2B), with the $\tau$ versus voltage relation unaltered. The amplitudes of the relaxations were also unaffected. This experiment was repeated in two other cells and with two myoballs perfused with 1 m*M* QX-222. In each case the ACh-induced current was unaffected.

If voltage could drive internal QX-314 to a binding site, the blocking action would be most apparent at positive potentials. Internal QX-314 had no noticeable effect on voltage jump relaxations at 40 mV. However, at positive potentials the relaxations were not readily resolved, due primarily to their small amplitudes (*2*). The steady-state currents at positive potentials, however, should reflect the equilibrium voltage dependence

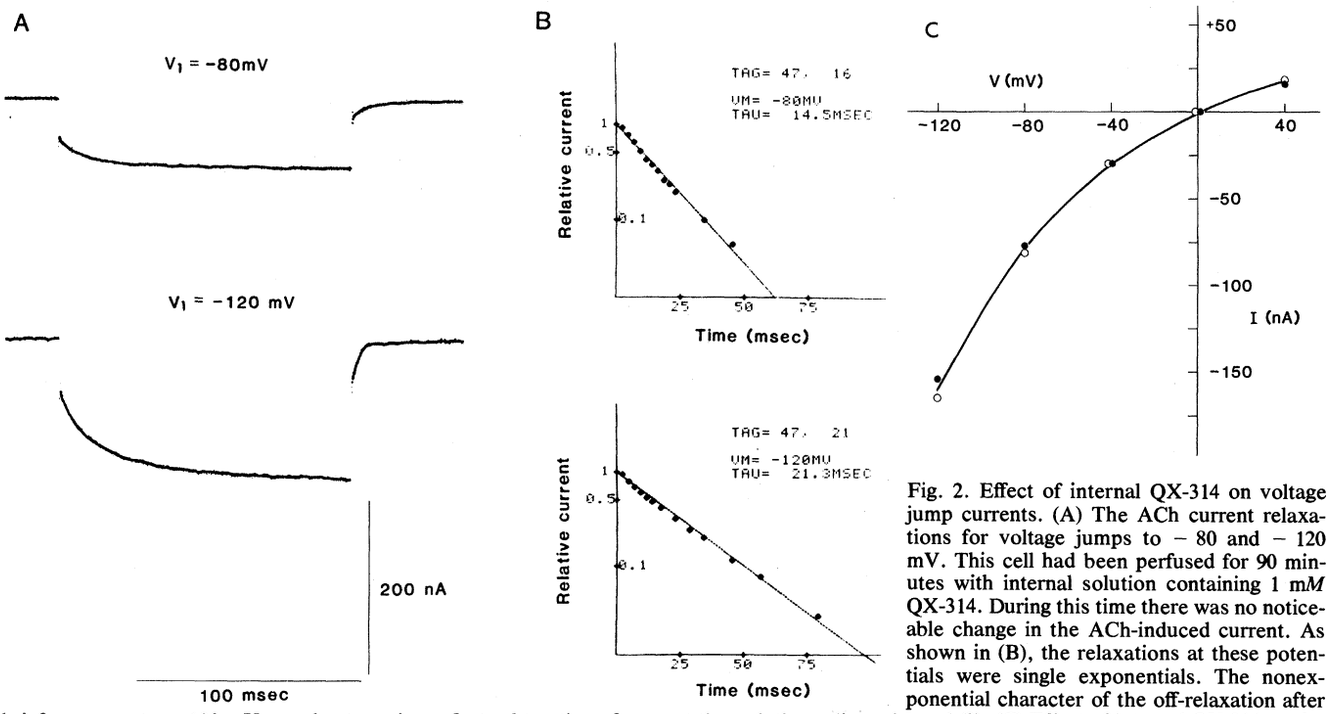


Fig. 2. Effect of internal QX-314 on voltage jump currents. (A) The ACh current relaxations for voltage jumps to − 80 and − 120 mV. This cell had been perfused for 90 minutes with internal solution containing 1 m*M* QX-314. During this time there was no noticeable change in the ACh-induced current. As shown in (B), the relaxations at these potentials were single exponentials. The nonexponential character of the off-relaxation after brief exposure to − 120 mV was due to an imperfect subtraction of current through the sodium channel (*2*). (C) Effect of internal QX-314 on the steady-state current-voltage relation [from the same cell used in (A) and (B)]. The iontophoretic current (*I*) was adjusted to give a − 30-nA response at the holding potential (− 40 mV). The circles indicate steady-state ACh-induced current 130 msec after voltage jumps to − 120, − 80, 0, and 40 mV [(○) control responses; (●) responses after 90 minutes of internal perfusion with 1 m*M* QX-314]. In both cases the current-voltage relation shows a pronounced curvature due to the voltage dependence of the lifetime of the open channel (temperature, 18°C) (*4*).

of the anesthetic block (*6*, *7*). These currents were also not influenced by internal anesthetics (Fig. 2C). This is in contrast to the effects of external anesthetics, which cause a marked alteration of the steady-state current-voltage relation for ACh-induced current (*6*, *7*).

To determine whether the anesthetics reached the internal membrane surface, the voltage-activated sodium current was examined in two myoballs. At a holding potential of − 80 mV, a 30-msec pulse to − 40 mV elicited an inward sodium current. In the absence of anesthetic compounds, the sodium current was not affected by stimulating at a frequency of 1 Hz. However, 20 minutes after the addition of 1 m*M* QX-314 to the internal solution, the sodium current exhibited a use-dependent block, decreasing by about one-third in response to 30 pulses at 1 Hz. Extracellular 1 m*M* QX-314 was without effect on the current through the sodium channel. A use-dependent inhibition of sodium current by internal QX-314 was reported previously (*9*).

It is possible that an asymmetry of surface potentials could explain the asymmetrical effects of QX-314 and QX-222 on the ACh channel. If the extracellular negative surface potential is greater than the intracellular surface potential, it could concentrate the cationic anesthetic at the outer mouth of the ACh channel, thus increasing its effectiveness when applied externally. If the extracellular surface charge density is about 0.002 electron charge per square angstrom (*10*), then the surface potential can be calculated by use of the Grahame equation (*10*, *11*). The calculated extracellular surface potential, − 41 mV, would increase the concentration of the monovalent cation from 0.1 m*M* in the bath to 0.5 m*M* at the outer membrane surface. This concentration is still less than the intracellular concentration, 1 m*M*. In spite of the fact that the intracellular concentration is greater, these anesthetics have no effect when applied intracellularly. It seems to us, therefore, that asymmetry of surface potential cannot explain this result.

Extracellular anesthetics appear to block channels by hopping over an extracellular barrier and binding to an intrachannel site within the transmembrane electrical field. Apparently the blocker is unable to hop over an even larger intracellular barrier to reach the inside of the cell. If the blocker could be driven by voltage over an intracellular barrier, the block would be relieved at sufficiently negative potentials by a "punch-through" mechanism (*12*), which has not been observed for the ACh channel (*6*, *7*). The large intracellular barrier may prevent access of internally applied anesthetics to the binding site. It is conceivable that the intracellular barrier is the selectivity filter of the channel (*2*). Regardless of the mechanism of action of anesthetics, it is clear from these experiments that the channel is not a symmetrical structure. Our data are in agreement with recent morphological evidence showing that the ACh receptor-channel complex has an asymmetrical structure within the lipid bilayer (*13*).

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25 January 1980; revised 17 April 1980

## Preserved Learning and Retention of Pattern-Analyzing Skill in Amnesia: Dissociation of Knowing How and Knowing That

Abstract. *Amnesic patients acquired a mirror-reading skill at a rate equivalent to that of matched control subjects and retained it for at least 3 months. The results indicate that the class of preserved learning skills in amnesia is broader than previously reported. Amnesia seems to spare information that is based on rules or procedures, as contrasted with information that is data-based or declarative—"knowing how" rather than "knowing that." The results support the hypothesis that such a distinction is honored by the nervous system.*

Amnesia, a neurologic syndrome characterized by a deficit in the formation of new memories, can exist independently of other cognitive impairment. The deficit is global, affecting both verbal and nonverbal material irrespective of modality (*1*). In particularly severe amnesia, as exhibited, for example, by the noted case of H.M. (*2*, *3*), the impairment has been described as "forgetting the incidents of daily life as fast as they occur" (*2*, p. 15).

Amnesic patients have nonetheless been reported to learn and remember certain perceptual-motor skills, including tracking and mirror tracing (*4*–*7*), frequently at a rate comparable to that of control subjects (*6*, *7*). Yet these same patients had little or no recollection of having previously performed the task.

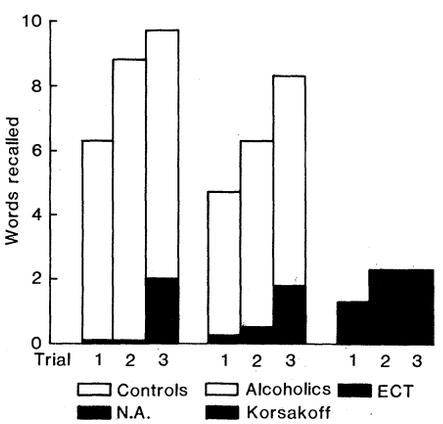


Fig. 1. Anterograde amnesia for ten word pairs presented three times. After each presentation, subjects saw the first word of each pair and tried to recall the second.


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