deficiencies of individual complement components have been described in animals. These have included C4 deficiency in the guinea pig (*14*) and rat (*15*), C5 deficiency in the mouse (*16*), and C6 deficiency in the rabbit (*17*). Although pharmacologic agents, such as cobra venom factor (*18*), have been used as an experimental tool for the study of C3 in vivo, the resulting C3 depletion is neither complete nor sustained. The availability of animals with C3 deficiency will permit the study of the immunobiology of C3 in vivo.

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Gallamine Triethiodide (Flaxedil): Tetraethylammonium- and Pancuronium-Like Effects in Myelinated Nerve Fibers

Abstract. *Gallamine triethiodide (Flaxedil) is commonly used as a neuromuscular blocking agent. Voltage-clamp studies show that gallamine also directly affects amphibian and mammalian myelinated nerve fibers. Externally, gallamine is about five times more potent than tetraethylammonium in blocking potassium conductance, where this is present, but has no effect on the sodium channel. Internal application slows sodium inactivation, which in addition is often incomplete. At positive potentials, gallamine can occlude sodium channels, thereby almost eliminating outward sodium currents.*

Since its introduction in 1947 (*1*), gallamine triethiodide (Flaxedil) has become one of the most commonly used neuromuscular blocking agents. The widespread use of gallamine, especially in neurophysiological studies, has depended in part on the belief that gallamine has no direct effect on nerve. However, the voltage-clamp studies described here reveal that externally applied gallamine has a potent blocking effect on potassium channels, where these are present, in both mammalian and amphibian nerve fibers. The blocking effect is sufficiently great that gallamine may have a direct effect on neural activity in gallamine-paralyzed preparations. Internal application of gallamine also has significant effects on the sodium channel; these effects resemble those produced by pancuronium bromide in squid giant axons (*2*).

Frog and rat myelinated nerve fibers were dissected and voltage-clamped by conventional methods (*3–5*). The nodes were held at potentials of −80 to −85 mV by adjusting for a steady-state inactivation of 0.8, and a 50-mV hyperpolarizing prepulse was applied to remove resting inactivation. Leakage and capacity currents were subtracted by an analog circuit, and membrane currents were re-

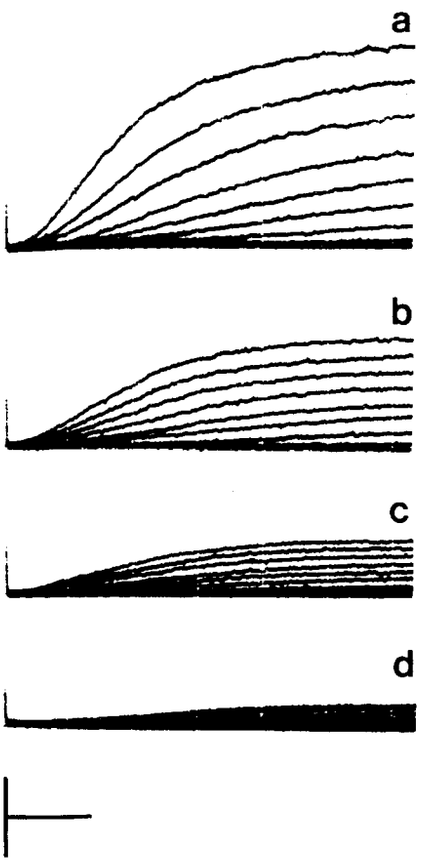

Fig. 1. Inhibition of potassium currents by gallamine in a frog fiber initially treated with $10^{-6}M$ tetrodotoxin to block the sodium conductance. (a) Control records in Ringer solution (115 m*M* Na^+; 2.5 m*M* K^+; 1.8 m*M* Ca^{2+}; 5 m*M* tris) for step depolarizations of 30 to 110 mV in 10-mV increments. (b to d) Records for the same depolarizations as in (a) but recorded 10 minutes after the addition of 0.1 m*M* (b), 1.0 m*M* (c), and 10.0 m*M* (d) gallamine triethiodide to the node in the A pool of the voltage-clamp chamber [see (*3*)]. Leakage current has been eliminated by the use of an analog circuit adjusted to null out those currents flowing during a 100-mV hyperpolarizing step. Calibrations are 8 nA and 2 msec. Holding potential, −85 mV; temperature, 15°C.

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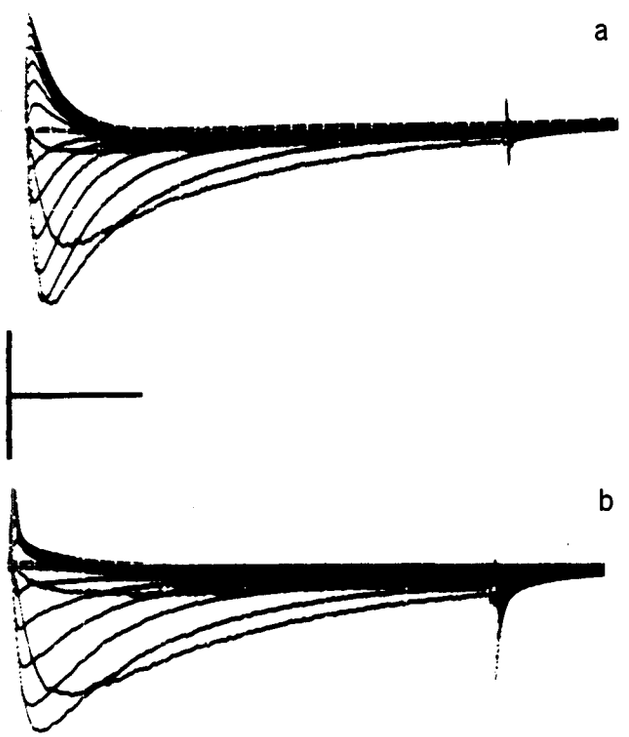
corded digitally on magnetic tape for later analysis. Sodium permeability (P_{Na}), potassium conductance (g_K), and membrane currents were calculated by standard methods (*3*). In the voltage-clamp chamber there were four saline-filled pools separated by narrow Vaseline-filled gaps. The end pools, containing the cut ends of the fiber, are termed C and E; the node was in A, and the adjacent internode was in B [see (*3*)]. Gallamine triethiodide was applied either externally in the Ringer solution bathing the node in the A pool, or internally by way of diffusion from the cut ends of the fiber which were bathed in isotonic KCl.

In frog nodes bathed in normal Ringer solution, application of 1 m*M* gallamine to the A pool significantly prolonged the nodal action potential, an effect seen under voltage clamp to be due to block of the delayed g_K (Fig. 1). The decrease in g_K averaged 60 percent at 0.1 m*M*, 77 percent at 1.0 m*M*, and 87 percent at 10.0 m*M* gallamine. The 10 to 15 percent residual, gallamine-insensitive potassium conductance was also resistant to tetraethylammonium (TEA^+), and thus may represent a voltage- and time-dependent leak. If this residual conductance is ignored (it was too small and variable to analyze), the dose-response data are consistent with a model in which a single gallamine molecule blocks a single potassium channel with an effective dissociation constant of $10^{-4}M$.

Most rat fibers examined did not show an appreciable g_K (*5, 6*) and, in such fibers, 10 m*M* external gallamine had no effect. However, in some instances a potassium conductance was present upon initial examination, and in all fibers a potassium conductance could be readily induced by adding 1 mg of lysophosphatidyl choline (LPC) per milliliter to the A pool for 15 to 45 seconds (*6*). In such fibers, external gallamine greatly reduced the potassium conductance, although the LPC-induced g_K was less sensitive to gallamine than either the g_K of frog fibers, or the g_K initially present at some rat nodes. When applied internally, 10 m*M* gallamine also blocked the potassium conductance in frog fibers but had no effect on g_k in rat fibers, whether present initially or induced with LPC.

Whereas external gallamine (up to 10 m*M*) had no effect on the sodium conductance in either frog or rat fibers, internal application of the drug had at least two distinct effects (Figs. 2 and 3). First, gallamine slowed sodium inactivation in fibers in which K^+ currents were either eliminated by TEA^+ (frog), or were naturally absent (rat). In mammalian fibers this effect could reasonably be described as a shift in the voltage dependence of Na^+ inactivation by 10 to 20 mV in the depolarizing direction, whereas for frog fibers gallamine was less effective for depolarizations near threshold. Even with long depolarizing pulses, a significant fraction of the sodium channels were not inactivated at positive potentials. This effect was particularly noticeable in rat fibers (Fig. 3). Thus, after repolarization there were large inward Na^+ tail currents, which in rat fibers also decayed sufficiently slowly so that they could be easily separated from the capacity transient.

The second major effect of internally



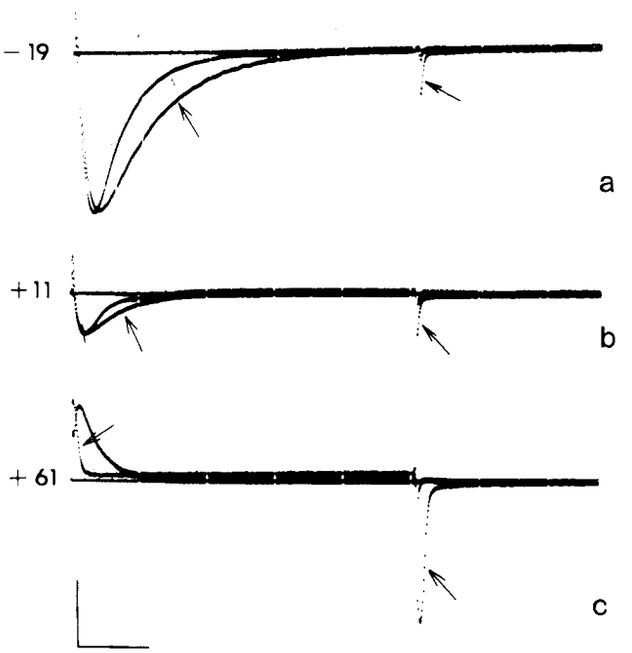


Fig. 2 (left). Effects of internally applied gallamine in a frog fiber initially treated with TEA^+ to block the potassium conductance. (a) The fibers were placed in Ringer solution containing 50 m*M* TEA^+ for voltage steps of 30 to 170 mV in 10-mV increments. (b) Records obtained 20 minutes after the addition of 10 m*M* gallamine to the isotonic KCl solution bathing the cut ends of the fiber in the C and E pools [see (*3*)]. Note that inactivation is not greatly affected for the two lowest depolarizations but is clearly slowed for the other inward currents. Outward currents are blocked, and the amplitude of the tail currents is slightly increased. Leakage currents have been subtracted as in Fig. 1. Calibrations are 10 nA and 3 msec. Holding potential, −87 mV; temperature, 15°C. Fig. 3 (right). Effects of internal gallamine on a rat myelinated fiber. In this case TEA^+ was not used since the fiber lacked a detectable potassium conductance. Control records and records obtained 15 minutes after addition of 10 m*M* gallamine to the C and E pools (the latter indicated by arrows) are superimposed for depolarizations to the indicated potentials. Note the slowing of sodium inactivation for the records at −19 mV (a) and +11 mV (b) and the block of outward current at +61 mV (c). The sodium tail currents are significantly increased (arrows) by gallamine, particularly following large depolarizations, and the currents decline more slowly than if the fiber had been repolarized in the absence of drug prior to the onset of Na^+ inactivation. Calibrations are 5 nA and 1.5 msec.

applied gallamine was to eliminate outward sodium currents almost completely, suggesting that the open sodium channels were occluded by the positively charged gallamine molecules. Occasionally, the combination of slowed inactivation and occlusion could be seen as a rapid initial decline in inward current, followed by a later decay that was slower than Na^+ inactivation in the control records. This effect was particularly noticeable at potentials near the Na^+ equilibrium potential. In general, gallamine did not change the time course of sodium activation or maximum sodium permeability. The nodal action potential following internal application of gallamine was prolonged by two to three times, as might be expected from the voltage-clamp results.

In addition to gallamine, we examined the effects of internally and externally applied acetylcholine, carbamylcholine, and *d*-tubocurarine. No effects on sodium permeability were seen at concentrations up to 10 m*M*. At this high concentration, acetylcholine and carbamylcholine (but not *d*-tubocurarine) slightly reduced the potassium conductance (10 to 20 percent), presumably because of their structural resemblance to TEA^+. Since gallamine is effective at much lower concentrations, its action is likely to be reasonably specific.

The actions of gallamine on the potassium channel resembled those produced by TEA^+ (*7–9*), except that gallamine was approximately five times more potent than TEA^+. In most respects, the effects of internal gallamine on the sodium channel mimicked those of another neuromuscular blocking agent, pancuronium bromide (*2*), although gallamine did not decrease maximum sodium permeability. Although gallamine did not affect the sodium channel acutely, this may not remain true with long-term exposure to the drug.

The high affinity of gallamine for the potassium channels of nerve membranes may have significant consequences in gallamine-paralyzed preparations. Particularly in neurophysiological studies, total paralysis is often required. The minimum paralyzing concentration (calculated on the assumption that the extracellular fluid is approximately 20 percent of body weight) of gallamine in human, cat, and rat are approximately 19 μM, 11 μM to 25 μM, and 69 μM, respectively (*10–12*), and concentrations up to ten times these levels are occasionally employed. The voltage-clamp data show that even 10 μM gallamine produces a detectable effect on g_K. Indeed, at concentrations lower than those required for neuromuscular block, gallamine has been shown to effect repetitive firing in both mammalian muscle and amphibian nerve fibers (*13–15*). The entry of gallamine to the central nervous system in intact preparations is impaired by the blood-brain barrier; but, particularly in experimental studies, this barrier is often breached by surgical or pathological processes. Consequently, it is important to note that while the myelinated axons of the mammalian central and peripheral nervous systems have few exposed potassium channels (*16*), their central synaptic endings are significantly affected by potassium-blocking drugs (*17*). Thus, one would expect gallamine, upon gaining access to the central nervous system, to have an excitatory effect on central nerve activity commensurate with its potassium-blocking property, and such an effect has been reported (*18*).

In view of the potent effects of gallamine on neural potassium and sodium channels in mammalian and amphibian nerve fibers, its continued use as a specific neuromuscular blocking agent should be reexamined, especially in circumstances where the blood-brain barrier may be disrupted by surgical or pathological processes.

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A Novel Illusion of Bars Made from Triangles

Abstract. *A repetitive pattern of a triangular luminance profile may be perceived as a triangular-wave grating or as a square-wave grating. This illusion may reflect the operation of cortical phase-selective mechanisms that are biased toward particular phase relations and favor abrupt luminance gradients.*

Visual illusions can provide insights about the processing of information by the visual system. Presumably these perceptual errors highlight neural operations that normally escape notice during everyday visual experience. Recently we happened to discover a novel illusion produced by viewing a one-dimensional grating pattern with a triangular spatial luminance profile (Fig. 1). Rather than maintain its initial appearance, the display soon became multistable—the percept alternated between a true triangular-wave grating and what appeared to resemble more closely a square-wave grating (Fig. 2) of the same period. We then inquired about the conditions necessary for experiencing this illusory square-wave grating and considered potential reasons for its occurrence.

The triangular-wave grating in Fig. 1 was generated electronically on a cathode-ray tube (CRT) display (*1*). Once perceived, the illusory square-wave grating remains present for several seconds before reverting to the genuine triangular wave. Typically, the alternations of these two distinct percepts resemble the reversals of a Necker cube. As a rule, each luminance peak and trough in the triangular-wave grating becomes the edge of an illusory bar. The illusion itself is bistable in that the light bars may appear either to the left or to the right of the luminance peak (Fig. 2, C and D); as a result, the illusory square-wave grating may appear to shift in phase (*2*). Some observers have spontaneously reported that the bars of the illusory square-wave grating often appear to lie in different

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