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## Curare and Pancuronium Compared: Effects on Previously Undepressed Mammalian Myoneural Junctions


Abstract. *Curare and pancuronium have multiple effects on previously undepressed rat diaphragm; these include depression of transmitter output and prolongation of the refractory period of prejunctional structures. The effect of curare on motor nerve terminals is greater than that of pancuronium. Both drugs depress postjunctional receptors; but curare, in addition, raises the threshold for the generation of muscle action potentials. In addition, these results raise questions about the validity of statistical methods used to calculate transmitter output.*


The principal site of action of curare (tubocurarine) at the myoneural junction is a matter of controversy. Some investigators consider that this alkaloid blocks neuromuscular transmission exclusively by occupying postjunctional cholinergic receptors (*1*). Others consider that, in addition, curare significantly depresses the release of transmitter from motor nerve terminals (*2*), and furthermore, that this depression represents its principal effect (*3*). This controversy can be partially explained by the pitfalls of the techniques used to analyze pharmacologic effects at the myoneural junction.

When acetylcholine release from muscle preparations during stimulation of motor nerves is used as evidence for a pre- or postjunctional site of action of curare, acetylcholine release is either unchanged (*4*) or reduced (*5*) in curare-treated preparations as compared to controls. These results reflect disparity in stimulation frequency and assay techniques. However, most arguments against the prejunctional effect of curare are based on the lack of effect of this drug on transmitter output, as supported indirectly by calculations derived from the quantal hypothesis of transmitter release. These calculations may be misleading because transmitter output is generally estimated in already paralyzed muscles (*6*)—or, if this output is not depressed, by using a correction factor for nonlinear summation of quanta (*7*). The application of this correction factor when potential changes are measured requires exact determination of the reversal potential for the transmitter at each end plate, as well as precise electrode localization (*8*). Moreover, this correction does not take into consideration temperature or type of muscle, two factors that affect the release and the postjunctional action of the transmitter. In undepressed muscle, contractions are prevented by cutting or treating the muscle with glycerol (*9*), treatments that affect transmitter output determinations (*8, 10*). Finally, even if these limitations can be avoided, the probability of release may be too high ($>.1$) (*11*) to apply Poisson statistics to calculate the quantal content of the end plate potential (EPP). This type of calculation may overestimate the quantal content by 100 percent if the probability changes from .1 to .5 (*12*).

Table 1. Probability of transmitter release at 32° ± 1°C and coefficients of variation of EPP amplitude. The concentration of drug was $5 \times 10^{-6} M$. Standard deviations are in parentheses (C, curare; P, pancuronium).

| Drug | Coefficient of variation | | Probability of transmitter release |
|---|---|---|---|
| | 0.01 to 1 hz | 1 to 10 hz | |
| None | 0.0196 (± 0.006) | 0.0315 (± 0.0078) | 0.044 (± 0.009) |
| C | 0.1012* (± 0.035) | 0.122* (± 0.0417) | 0.15* (± 0.077) |
| P | 0.0863* (± 0.014) | 0.0925* (± 0.030) | 0.18* (± 0.098) |

* $P < .01$ compared to control.

In the experiments reported here, previously untreated muscles were used to determine dose-response curves of curare on the amplitude of the EPP and strength of contractions. The effects of curare were compared with those of pancuronium bromide, a steroid with neuromuscular blocking characteristics said to be identical to those of curare (*13*). This steroid has biomedical importance because it lacks the ganglion-blocking and histamine-releasing properties of curare (*14*) and has been proposed as a substitute for curare in clinical anesthesia. The use of Poisson statistics to determine the effect of curare at the myoneural junction was shown to be inaccurate, because curare increased the probability of transmitter release above .1.

A new preparation was developed in this laboratory (*15*). Rat diaphragms were stretched to permit intracellular recording during isometric contractions. Observations with a variety of drugs, in which the new technique was compared with conventional methods, indicated that each compound had a characteristic pattern of neuromuscular depression independent of the experimental preparation (*16*).

Dose-response curves for the effects of both muscle relaxants on strength of muscle contraction and EPP amplitudes were made at 32° ± 1°C (Fig. 1). Although the EPP depression was similar with both drugs, their actions on contraction strength were different, as seen by the steeper response curve with pancuronium. Curare had a greater effect on muscle contractions for a similar depression of the EPP; this suggests that, in addition to depressing EPP amplitude, curare directly affected either the contractility or the threshold for the propagation of EPP to the muscle fiber.

Because the contractility of directly stimulated muscles was not depressed by this drug, curare may have raised the threshold of EPP propagation. This possibility was confirmed by comparing the minimal EPP's that could initiate muscle action potentials when each muscle relaxant was used. The EPP amplitude was determined at the moment when propagation to the muscle fiber either ceased in response to the drug, or appeared after the drug was withdrawn from the bath. Only EPP's above 4 mv, at a resting membrane potential more negative than −60 mv, were propagated in the presence of curare, while approximately 2-mv depolarization was needed to trigger muscle action potential when pancuronium was used. There were no significant changes in the rate of rise of the EPP. The threshold with curare was lower than the 10 mv reported for mammalian muscles treated with curare (*17*). The possibility that pancuronium lowers the threshold is remote, if it is assumed that this drug acts on muscle fibers as it does on motor nerve terminals.

Both muscle relaxants increased the absolute refractory period of motor nerve terminals. This period (± standard deviation) was 0.96 ± 0.1 msec in controls, 2.3 ± 0.48 msec in the presence of $5 \times 10^{-6} M$ curare ($P < .01$), and 1.4 ± 0.92 msec in the presence of $5 \times 10^{-6} M$ pancuronium (difference not significant). This period was determined by delivering paired stimuli of at least twice threshold strength to the phrenic nerve; the interval between pulses for which no EPP could be evoked after the second pulse was considered the absolute refractory period. It was expected that a postjunctional depression would have a stepwise effect on EPP amplitude and not the observed all-or-nothing type of response. Frequency of miniature EPP's, which reflects the function of motor nerve terminals (*18*), was not reduced by subparalytic doses of pancuronium (*19*) but was depressed by low concentrations of curare (*15*).

Table 1 presents calculations of probability of transmitter release and the coefficient of variation of EPP amplitude. The probability was obtained from the slope of the second to eighth EPP's evoked at 100 hz (*20*). When curare or pancuronium was given, the probability became greater than .1, as calculated from the faster reduction in EPP amplitude during tetanic stimulation. (It is assumed that these drugs do not desensitize the end plate to the action of acetylcholine.) Curare and pancuronium significantly raised the coefficient of variation, but quantification of transmitter output was not intended because it was not possible to determine the reversal potential for the transmitter at every end plate investigated. However, it is reasonable, according to the quantal hypothesis, to suggest that a larger coefficient of variation in addition to a higher probability of release indicates a significant depression of transmitter output. Effects on the refractory period at motor nerve terminals support this suggestion. Experimental conditions that affect only the postsynaptic membrane, such as changes in the resting membrane potential, had no effect on the coefficient of variation of EPP amplitude distribution (*21*). On the other hand, prejunctional effects, such as those induced by high frequency of stimulation greater than 1 hz, increased the value of the coefficient (Table 1).

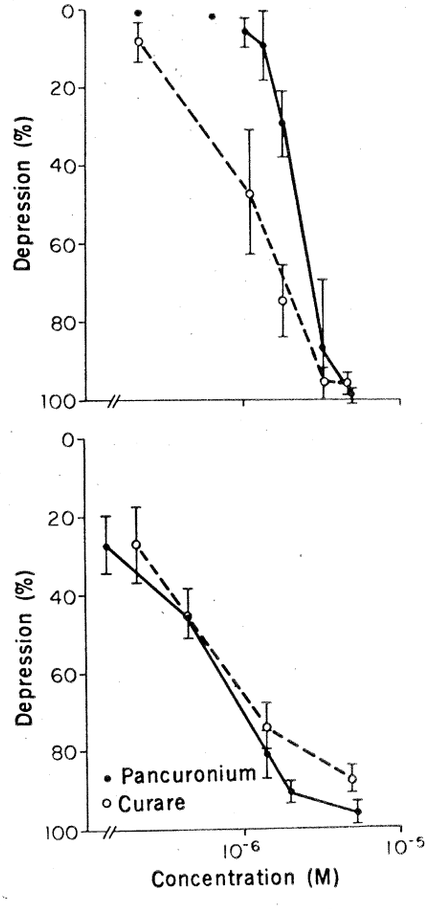


Fig. 1. Dose-response curves for curare and pancuronium. Effects on the strength of indirectly evoked muscle contractions are shown in the upper graph and the amplitude of the EPP is shown below. Each point is the mean of at least five muscles or ten EPP's (bars, standard deviation). The temperature was 32° ± 1°C, and measurements were made 15 to 30 minutes after equilibration of the bath.

The objective of these experiments was to compare the effects of curare and pancuronium bromide on neuromuscular transmission of previously undepressed rat diaphragms, and to raise some questions on the validity of present statistical methods used to calculate transmitter output. Curare not only acts on postjunctional receptors, but must significantly depress prejunctional factors responsible for the amplitude of the EPP. Furthermore, much of the neuromuscular blockade caused by curare is mediated by an increase in the threshold of the muscle fiber for the propagation of EPP's as action potentials. Prejunctional structures are more depressed by curare than by pancuronium, while the latter appears to act exclusively on synaptic components. The statistical analysis of EPP amplitude distribution, although supporting a significant prejunctional effect by these two muscle relaxants, is of little value to determine pharmacologic effects on transmitter output.

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21. The coefficient of variation was calculated from more than 150 EPP's measured at the same (± 2 mv) resting membrane potential. These observations were made at resting membrane potentials ranging from 36 to 51 mv.
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## Ionic Basis of the Photoresponse of Aplysia Giant Neuron: $K^+$ Permeability Increase

Abstract. *The giant neuron of the* Aplysia *abdominal ganglion hyperpolarizes during illumination. The light-initiated potential change is associated with an increase of membrane conductance. It reverses sign at the potassium equilibrium potential (about −83 millivolts), which was determined from direct measurements of internal potassium activity. The membrane hyperpolarization is produced entirely by a light-induced increase in potassium permeability.*

The ionic basis of the depolarizing receptor potential has been examined in detail in photoreceptors of *Balanus, Limulus*, and *Apis* (*1*). However, the ionic basis of hyperpolarizing receptor potentials in vertebrate and invertebrate photoreceptors has been more difficult to determine (*2, 3*). We studied the light-induced membrane hyperpolarization in the giant neuron of the abdominal ganglion of *Aplysia californica* (*4*), and found that it was due exclusively to an increase in membrane permeability to potassium ions.

The giant neuron was illuminated with white light from a tungsten-halide source or with monochromatic light from a mercury-xenon source. Techniques of voltage and current clamp and the measurement of membrane potential and internal $K^+$ activity have been described (*1, 5*).

About 400 msec after the giant cell was illuminated with a pulse of white light, the membrane gradually hyperpolarized, reaching its most negative value in about 20 seconds (Fig. 1A). There was usually a slight decline in the potential change during sustained illumination. When the light pulse was terminated, the membrane repolarized, requiring about 40 seconds to attain the resting level prior to illumination. Occasionally there was an even slower decay phase (Fig. 1B). The membrane potential change is smaller and slower than the potential change to light that has been described for other photoreceptors. Spectral studies indicated that the potential change is elicited most effectively at 490 nm. The membrane potential changed when constant-current pulses were passed across the membrane prior to, during, and after illumination (Fig. 1B). Light produced a membrane hyperpolarization of about 5 mv. The input resistance of the membrane decreased from 1.58 megohms in darkness to 1.38 megohms during illumination. This represented an increase of membrane conductance of $10^{-7}$ ohm$^{-1}$, or 13 percent over that in darkness. Figure 1C shows a record of light-elicited membrane current when the membrane potential was voltage-clamped to the resting potential. Light elicited a slowly changing outward membrane current with about the same time course as the membrane potential change (see Fig. 1A). The similarity in waveforms is to be expected since the time constant of the membrane (about 180 msec) is much shorter than the change in membrane current.

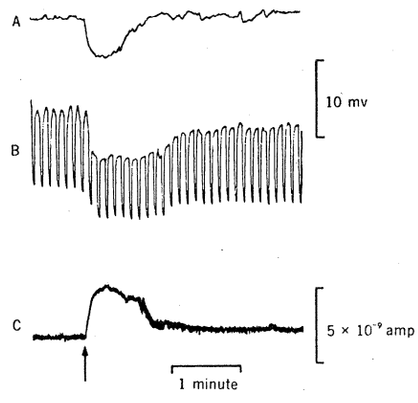


Fig. 1. (A) Membrane potential change produced by illumination of the *Aplysia* giant neuron. Resting potential was −45 mv. (B) Potential changes produced by passing 6-na current pulses (inward) across the giant neuron prior to, during, and after illumination. Resting potential was −45 mv. (C) Membrane current produced by light when the giant cell membrane was voltage-clamped to the resting potential (−54 mv). The onset of illumination is indicated by the arrow at the bottom of the traces. The periods of illumination were (A) 30 seconds, (B) 1 minute, and (C) 50 seconds.

The possibility that these changes in the giant cell are produced by synaptic action due to light stimulation of a presynaptic cell was ruled out. Low $Ca^{2+}$ (0.5 m*M*) solutions that abolish or greatly reduce synaptic action in this cell augmented, rather than diminished, the membrane potential change to light. Furthermore, the hyperpolarization to light was maintained in two cells that were ligated between the soma and the axon with a fine thread and completely removed from the ganglion. The possibility that the change is produced by light activation of an electrogenic pump was also ruled out. Ouabain or $K^+$-free solutions, which depolarized the cell immediately on application, presumably due to blockage of an electrogenic $Na^+$ pump (*6*), had little effect on the potential change to light at the time the pump was manifestly blocked.

The potential changes elicited by light at different membrane potential levels produced by passing steps of current of different intensities across the cell membrane was recorded (Fig. 2B). In each case, sufficient time was allowed during the passage of current for the membrane to attain a relatively steady potential level before light was applied. In control solutions (Fig. 2B), the light-induced potential change became smaller as the membrane was hyperpolarized, and at −81 mv there was a very small negative deflection to light. The sign of the potential change to light was reversed at membrane potential levels more negative than −84 mv. The relation between the maximum potential change elicited by light, $\Delta V_L$, and the membrane potential, $V_m$, prior to illumination is shown by the solid line in Fig. 2A. The line intersects the membrane potential axis at about −84 mv. The potassium equilibrium potential ($E_K$) determined at the same time from measurements of the internal $K^+$ activity (186 m*M*) was −83.6 mv (*7*). In 11 other cells, the differences between the mean value of the reversal potentials and the mean value of the simultaneously determined $E_K$'s was 1.3 mv. The potential changes to light at different membrane potential levels were measured when the external $K^+$ activity was raised to 21 m*M* (concentration, 30 m*M*). Immediately after the solution was changed, the resting potential became more negative and the