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Procaine alters fast excitatory postsynaptic current decay in amphibian sympathetic ganglia

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1 The effects of procaine (25–200 μM) on the fast excitatory postsynaptic currents (e.p.s.cs) of bullfrog sympathetic ganglion B cells were studied with a two-microelectrode voltage clamp system.

2 Procaine decreased peak e.p.s.c. size with no measurable decrease in quantal content.

3 The peak e.p.s.c.-voltage relationship was linear in control cells, but showed a marked nonlinearity in cells treated with procaine so that peak e.p.s.c. size decreased progressively at hyperpolarized values of membrane potential. The e.p.s.c. reversal potential was not altered in procaine.

4 Although the e.p.s.c. decay time course was well described by a single exponential in control cells, the decay phase often became complex in the presence of procaine. The decay phase consisted of two components in the presence of procaine which became more obvious with increasing concentration and membrane hyperpolarization.

5 In control cells, the e.p.s.c. time constant of decay increased with membrane hyperpolarization. In the presence of procaine, the first time constant of decay, τ_f, increased with hyperpolarization up to −40 mV, but then decreased with hyperpolarization between −40 and −100 mV.

6 We conclude that procaine has two sites of action at postganglionic sympathetic neurones: (1) it reduces the number of activatable receptor-channel complexes and (2) procaine blocks open synaptic channels. Blockade of open channels became more important with hyperpolarization.

Introduction

The local anaesthetic procaine alters transmission at cholinergic synapses in a number of different vertebrate and invertebrate preparations (Furukawa 1957; Maeno, 1966; Kordas, 1970; Deguchi & Narahashi, 1971; Katz & Miledi, 1975; Adams, 1977; Marty, 1978; Koblin & Lester, 1979). It has been suggested that at most of these synapses procaine interacts with and blocks the open state of the receptor-channel complex so that ionic flow through the synaptic channels is transiently interrupted (Adams, 1976; Ruff, 1976). Furthermore, the development of postsynaptic channel blockade by procaine appears to be the primary mechanism of action at many of these sites. In a recent study of the fast excitatory postsynaptic current (e.p.s.c.) in sympathetic ganglion cells, MacDermott, Connor, Dionne & Parsons (1980) found that the rate and voltage-dependence of e.p.s.c. decay were different from that of the muscle endplate current. This observation suggested that some of the kinetic characteristics of the acetylcholine-gated channel at ganglion cells differ from those at the motor endplate. To investigate further some of the basic pharmacological and kinetic properties of the ganglionic fast e.p.s.c., we have studied the alterations in ganglionic transmission, produced by procaine. We show here that, at sympathetic ganglia, procaine acts postsynaptically to reduce e.p.s.c. amplitude and alter the e.p.s.c. decay time course. These effects were both concentration- and voltage-dependent. Our results suggest that in the ganglion preparation procaine may act both by the blockade of open synaptic channels and by the reduction of the number of activatable receptor-channel complexes.

Methods

All experiments were done *in vitro* on B cells in the VIII, IX, and X ganglia from the paravertebral sympathetic chain of the bullfrog, *Rana catesbeiana*. The sympathetic chain from the 5th to 10th ganglia and the VIII, IX and X spinal nerves were excised and maintained in a temperature-controlled recording chamber mounted on the stage of a compound mic-

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roscope. The ganglion preparations were bathed in a buffered frog Ringer solution (composition in mM: NaCl 120, KCl 2.5, $CaCl_2$ 1.8, HEPES 1.0, pH 7.3, temperature 20–23°C). Concentrated stock solutions of procaine (procaine hydrochloride, Sigma Chemical Co., St. Louis, MO) were made weekly and diluted just before use. In each preparation, e.p.s.cs were obtained from a number of individual control cells and then from different cells after exposure to procaine. The results presented here represent currents recorded from 110 cells from 16 ganglion preparations.

The techniques used to record e.p.s.cs from individual ganglion cells were identical to those described previously by MacDermott *et al.*, (1980). Individual B cells were identified visually (150–200 ×) and voltage-clamped in the range − 100 to + 30 mV with a two microelectrode voltage clamp system. The voltage and current electrodes were each filled with 3 M KCl and had resistances ranging from 6–20 megohms when measured in Ringer solution. Voltage control during e.p.s.cs was considered acceptable only if the voltage deviation at the peak of the response was less than 0.5% of the driving force (i.e. membrane potential minus the e.p.s.c. reversal potential, $E_m - E_r$). The preganglionic fibres were stimulated at a constant frequency of 0.4 Hz in all experiments. Individual e.p.s.cs were recorded and stored on a PDP/8E or 11/03 laboratory computer (Digital Equipment Corp., Marlboro, MA). Three to ten e.p.s.cs were averaged at each voltage and subsequently analysed for peak current size and decay time course. The decay time course for all e.p.s.cs was fitted both as a single or a double exponential function by a computerized, non-linear least squares multi-exponential fitting programme (written by R. K. Wright, Department of Mathematics, University of Vermont).

Quantal content (m) was estimated both in control and procaine-treated preparations. Since ganglionic spontaneous miniature synaptic currents are smaller than the baseline noise of our voltage clamp, quantal content was estimated from measurements of the coefficient of variation (CV) of evoked e.p.s.cs (del Castillo & Katz, 1954). This method overestimates m at high levels of release (Martin, 1966). However, it can be used to compare estimates of m in control and drug-treated preparations to assess whether a presynaptic site of action contributes to any observed reductions in e.p.s.c. amplitude (Auerbach & Betz, 1971). To estimate the quantal content, a series of 60–200 e.p.s.cs were collected at a constant stimulation frequency of 0.4 Hz and analysed for peak current amplitude. The e.p.s.c. amplitudes were plotted as a function of sample number. A regression line was fitted to the linear portion of this plot and the variance σ^2) around the regression line was calculated using the Minitab statistics package (Penn. State Univ.). This procedure corrects for any progressive change in m due to depression or facilitation of transmitter release occurring during the sample period (Colomo & Rahamimoff, 1968). Quantal content was determined using the following relationship:

$$m = \frac{1}{(CV)^2}$$

where CV = σ/mean e.p.s.c. amplitude.

Data that represent the average of values obtained from several cells are expressed as mean ± standard error of the mean (s.e.mean).

Results

Procaine decreases e.p.s.c. amplitude

The peak amplitude of the e.p.s.c. was reduced in the presence of procaine. The effect of procaine concentration on peak current amplitude is illustrated in Figure 1. The amplitude of e.p.s.cs recorded at − 50 mV decreased progressively in the presence of 50–200 μM procaine. The reduction in amplitude was more dramatic over the same concentration range in cells voltage-clamped to − 90 mV. E.p.s.cs recorded in concentrations of procaine greater than 300 μM were very small at all voltages. As a result,

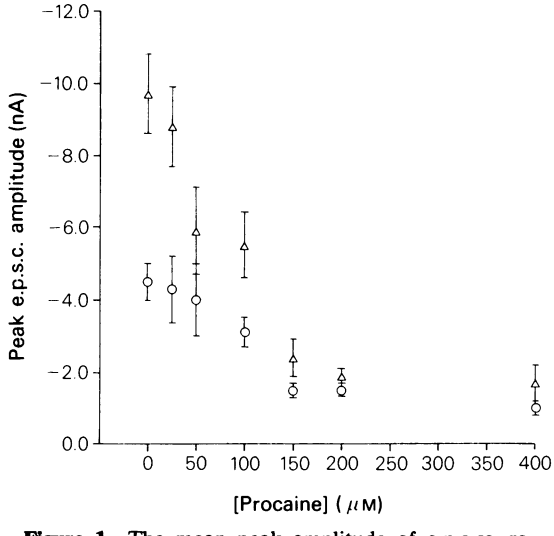

Figure 1 The mean peak amplitude of e.p.s.cs, recorded at −50 (○) and −90 mV (△) plotted as a function of procaine concentration. Each point is the mean value of e.p.s.cs recorded from at least three cells; s.e.means indicated by vertical lines.

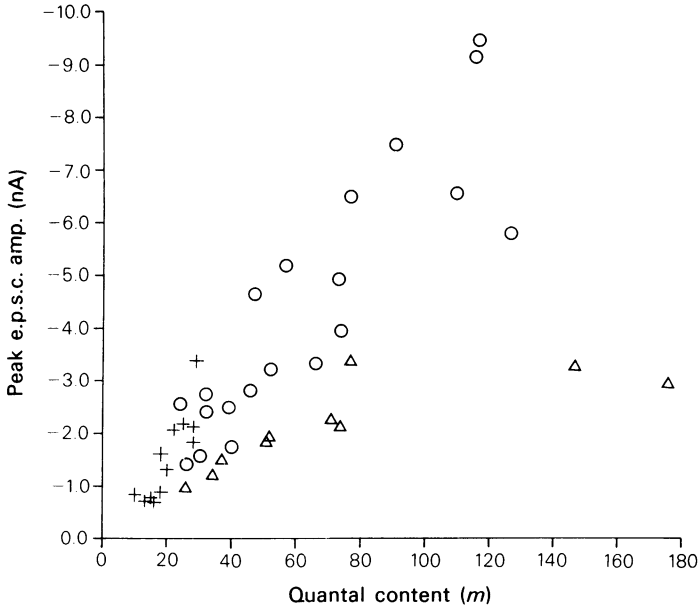

Figure 2 A plot of mean e.p.s.c. size versus quantal content estimated by the coefficient of variation method. All results were obtained in individual fibres voltage-clamped to −50 mV. (○) Represents data from cells maintained in Ringer solution containing 1.8 mM calcium; (+) shows data from cells maintained in Ringer solution containing 1.8 mM calcium and 8 mM magnesium, and (△) indicates data from cells exposed to 150 μM procaine.

our analysis of the action of procaine was limited to a concentration range of 25–200 μM.

Procaine does not alter quantal content

The decrease in e.p.s.c. amplitude produced by procaine could result from either a presynaptic or postsynaptic site of action. To determine if procaine acts presynaptically, the influence of procaine on the quantal content (m) was made from an analysis of the coefficient of variation of e.p.s.cs. In these experiments, cells were voltage-clamped to −50 mV and exposed to 150 μM procaine. This concentration was chosen because e.p.s.c. size was reduced significantly below that of controls, but a sufficient signal to noise ratio was maintained. Exposure to 150 μM procaine did not significantly alter quantal content (Mann-Whitney test). To insure that the CV method was sensitive enough to detect changes in m under our experimental conditions, we also tested the influence of magnesium, an agent known to decrease quantal content (del Castillo & Engbaek, 1954; Blackman, Ginsborg, & Ray, 1963). The addition of 8 mM magnesium reduced peak e.p.s.c. amplitude to approximately the same extent as 150 μM procaine. Furthermore, in the presence of magnesium, m was significantly decreased by 63% (Mann-Whitney test). These results are summarized in Figure 2.

We conclude that our method would have detected any significant alteration in quantal content by procaine if it had occurred. Consequently, the observation that in procaine-treated cells the e.p.s.cs size was reduced markedly without any observed change in quantal content suggests that procaine has primarily a postsynaptic site of action in sympathetic ganglia. The reduction in e.p.s.c amplitude could result postsynaptically from a shift in the e.p.s.c. reversal potential to a more negative value, a decrease in the number of activatable receptor-channel complexes, or open channel blockade. The remaining experiments were done to investigate which of these possible postsynaptic mechanisms contributes to the reduction in e.p.s.c. size produced by procaine.

The current-voltage relationship is non-linear in procaine-treated cells

The peak e.p.s.c.-voltage (I-V) relation of cells in control solution increases linearly with hyperpolarization in the voltage range between −20 and −100 mV (Kuba & Nishi, 1979; MacDermott *et al.*, 1980). In the present series of experiments, 26 of 27 control cells exhibited a linear dependence of peak e.p.s.c. amplitude on membrane voltage. A typical I-V plot for a control cell is shown in Figure 3. In procaine-treated cells, the peak current-voltage relation was often non-linear, exhibiting a marked decrease in peak current amplitude with hyperpolariza-

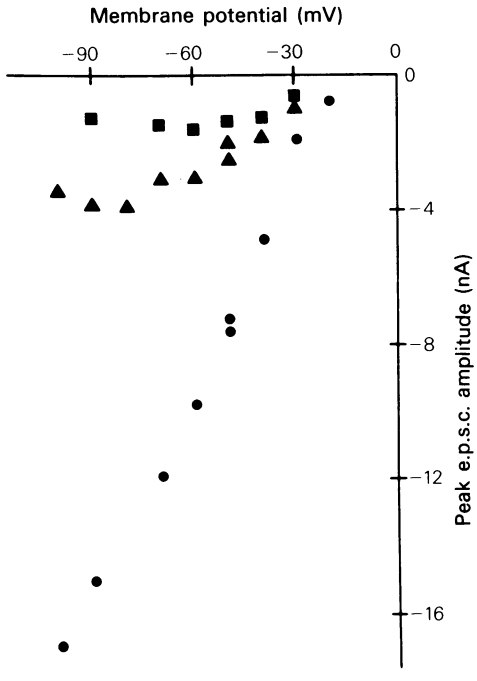

Figure 3 The current-voltage relation (I-V) for a control cell (●), is compared with the I-V relations for two procaine-treated cells (▲, 150 μM; ■, 200 μM).

tion. The percentage of cells exhibiting this deviation increased as a function of concentration. For example, in the presence of 50 μM procaine, 43% of the cells had non-linear I-V curves while at 100 and 200 μM procaine, this percentage increased to 57 and 83%, respectively. This non-linearity is illustrated in Figure 3 for two other cells exposed to 150 μM and 200 μM. Both the control cell and two procaine-treated cells were taken from the same ganglion preparation.

The reversal potential (E_r) of the e.p.s.c., determined by interpolation in cells in which e.p.s.cs were collected at positive membrane potentials, was not significantly changed by procaine treatment (Student's unpaired *t* test). The average value of E_r was -5.0 ± 1.0 mV in 24 control cells and -4.3 ± 2.0 mV in 5 procaine-treated cells (50–200 μM).

Procaine produces a voltage-dependent alteration in e.p.s.c. decay

The decay phase of control e.p.s.cs is well described by a single exponential (MacDermott *et al.*, 1980). The decay rate of the e.p.s.c. is independent of the size of the e.p.s.c., but is dependent on the membrane voltage (MacDermott *et al.*, 1980). The time constant (τ) of e.p.s.c. decay increases with hyperpolarization. This voltage dependence of τ can be described by

$$\tau(V) = a\,EXP\,(AV)$$

where a is τ(0) and A is the coefficient of voltage-dependence.

In most procaine-treated cells, the e.p.s.c. decay phase could not be adequately fitted by a single exponential; rather, the decay consisted of two exponential components. The complex decay became more apparent at high procaine concentrations and hyperpolarized values of membrane potential. In all instances, the amplitude of the second component was very small; being less than 15% of the total e.p.s.c. size. Because of its small size, the second exponential component has not been analysed further. A representative record, illustrating the pattern of complex decay in procaine-treated cells is shown in Figure 4. An e.p.s.c., obtained from a control cell, voltage-clamped to −90 mV, is shown in Figure 4a. The e.p.s.c. decayed as a single exponential with a time constant (τ) of 5.1 ms. Figure 4b is an e.p.s.c. recorded from another cell voltage-clamped to −90 mV and treated with 100 μM procaine. The decay phase of this e.p.s.c. was composed of two exponential components having time constants of 2.6 and 8.0 ms. In the present experiments we have quantitated the voltage and concentration-dependence of the initial decay time constant obtained in procaine-treated cells.

Procaine altered the voltage-dependence of e.p.s.c. decay. E.p.s.cs from control cells were prolonged at hyperpolarized potentials and therefore, exhibited a negative voltage-dependence. This voltage-dependence is summarized in the graph of Ln τ versus membrane voltage presented in Figure 5. The coefficient of voltage-dependence (A) for this control cell was $-0.0033\ mV^{-1}$. For 20 control cells the value of A was $-0.0045 \pm 0.0005\ mV^{-1}$. At membrane potentials more positive than approximately −50 mV the e.p.s.c. decay-voltage relationship was similar in procaine-treated cells to that of control cells. However, e.p.s.cs recorded from procaine-treated cells became progressively shortened with hyperpolarization in the voltage range between −40 to −100 mV. This complex voltage-dependence is illustrated in Figure 5. In this figure, the Ln of the initial decay component time constant for a cell exposed to 150 μM procaine is plotted as a function of membrane potential. For this cell, the e.p.s.c. duration increased with hyperpolarization from −30 to −50 mV and then progressively decreased when the cell was hyperpolarized further up to −100 mV. In the voltage range −50 to −100 mV, the coefficient of voltage-dependence of the first e.p.s.c. decay component was $+0.0052\ mV^{-1}$. A similar complex pattern of voltage-dependence was

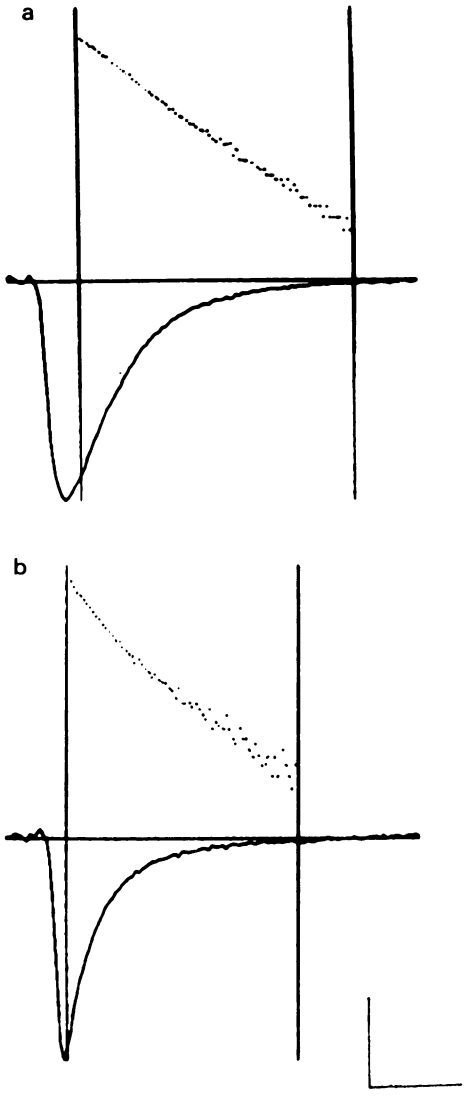

Figure 4 The influence of procaine on e.p.s.c. decay. The trace in (a) is from a control cell voltage-clamped to −90 mV. The peak amplitude was −11.6 nA and the time constant of decay was 5.1 ms. The trace in panel (b) was recorded at −90 mV from a cell treated with 100 μM procaine. The peak current was −6.3 nA and the two time constants of decay were 2.6 and 8.0 ms. The Ln of the current decay is shown above each trace. The vertical scale is 5 nA for (a) and 3 nA for (b), and the horizontal scale is 8 ms.

observed in 5 of 6 cells treated with 50 μM procaine, in 6 of 7 cells exposed to 100 μM and in all cells exposed to 150 μM or 200 μM procaine. However, the positivity of the voltage-dependence generally was greater in those cells exposed to 100 μM procaine

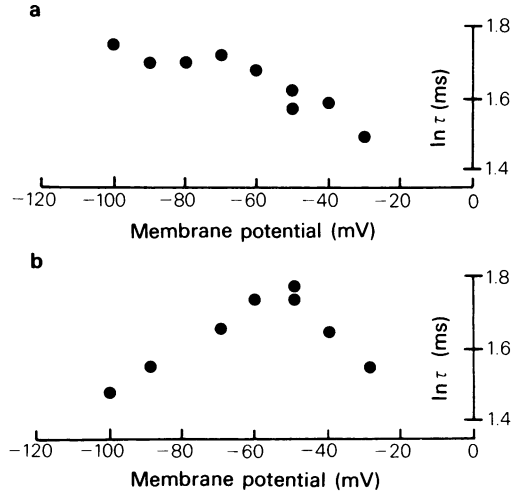

Figure 5 The influence of procaine on the voltage-dependence of e.p.s.c. decay. (a) the time constant (τ) of decay for a control cell is plotted as a logarithmic function of membrane voltage. (b) The voltage-dependence of τ_f, the initial decay component, is plotted for a cell treated with 150 μM procaine.

than to 50 μM procaine. For example, in the 5 cells exposed to 50 μM procaine, which exhibited a positive voltage-dependence of decay, the coefficient of voltage-dependence was $+0.0059 \pm 0.0021$. For comparison, in the 6 cells exposed to 100 μM procaine, which had the positive voltage-dependence of decay, the coefficient of voltage-dependence was $+0.0107 \pm 0.0027$.

The e.p.s.c. decay also shortened as the procaine concentration was raised. However, this concentration-dependence of procaine on the decay time course was most evident when the cells were voltage clamped to −90 mV and was not present to the same extent when the same cells were voltage-clamped to −50 mV. The effect of procaine concentration on the first decay time constant, τ_f, at both −50 and −90 mV, is summarized in Table 1. At −90 mV, τ_f was significantly decreased when the procaine concentration was raised from 50 μM to 100 μM (Student's unpaired t test, $P < 0.05$).

Discussion

Our results demonstrate that procaine reduced e.p.s.c. amplitude as a function of concentration without any measurable effect of quantal content. A lack of any significant presynaptic mechanism of action is similar to that reported previously for local anaesthetic action at the neuromuscular junction (Maeno, 1966; Kordas, 1970). In addition, procaine

Table 1 Voltage- and concentration-dependent alteration of e.p.s.c. decay by procaine

Treatment	$\tau_{-50}{}^{+}$ (ms)	$\tau_{-90}{}^{+}$ (ms)
Control	4.4 ± 0.1 (26)*	5.0 ± 0.1 (26)
Procaine 50 μM	4.4 ± 0.5 (7)	3.8 ± 0.4 (7)**
Procaine 100 μM	4.0 ± 0.3 (16)	3.1 ± 0.3 (13)**

+ Decay τ for single exponential in controls and initial decay time constant (τ_1) in procaine-treated cells.
* Mean ± s.e.mean (n).
** Significant difference, Student's unpaired t test: $P < 0.05$.

altered the kinetics of e.p.s.c. decay; this effect being sensitive to both drug concentration and membrane voltage. In the ganglion preparation as in other preparations procaine did not change the reversal potential of the e.p.s.c. so that the reduction in e.p.s.c. size was not due to an alteration of the ion selectivity of the channel.

The strong voltage-dependence of the procaine-induced alteration in the I-V relationship and decay time course at membrane potentials more negative than − 40 mV suggests that at least part of the action of procaine can be attributed to open channel blockade. The sequential model most commonly used to account for the characteristics of channel blocking drugs is the following:

$$nACh + R + Q \overset{K_D}{\longleftrightarrow} ACh_nR + Q \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} ACh_nR^* + Q \underset{F}{\overset{G}{\rightleftharpoons}} ACh_nR^*Q$$

where R represents the receptor-channel complex; ACh_nR^*, the open conducting state; Q, procaine; and ACh_nR^*Q, the blocked, non-conducting state (Adams, 1976; Ruff, 1977). At the neuromuscular junction, the splitting of endplate current decay by procaine is more obvious than that observed in sympathetic ganglion cells. However, the time constants of decay seen in the presence of a blocker are dependent on the value of the rate constants, G and F. A smaller unblocking rate constant, F, for ganglion cells would result in a second component of small amplitude, similar to those observed in these experiments. Koblin & Lester (1979) have reported an alteration of the decay phase of e.p.s.cs in electroplaque by procaine similar to that observed in these ganglion cells. These authors also suggest that the slow component is negligible in electroplaque because the unblocking rate constant, F, probably is significantly smaller in that preparation than in muscle.

A voltage-dependent decrease in e.p.s.c. amplitude and decay time course is consistent with the sequential blocking model. Therefore, the results obtained in cells voltage-clamped to − 90 mV are consistent with this class of mechanism. However, e.p.s.c size was also significantly decreased in cells voltage-clamped to − 50 mV in the presence of procaine concentrations (50–150 μM) which produced very little alteration in the decay time course. This observation suggests that procaine may also have a second mode of action which contributes to the decrease in e.p.s.c. size at less negative voltages. Two possibilities are that procaine interacts with agonist recognition sites or closed channels; either of which would lead to a reduction in the number of activatable receptor-channel complexes available for transmitter interaction.

We have also shown that e.p.s.c. decay is complex in atropine-treated cells (MacDermott *et al.*, 1980). The splitting of the e.p.s.c. decay by atropine is more dramatic and has somewhat different characteristics from that by procaine. In the presence of atropine, the time constant of the first decay component is concentration- but not voltage- dependent while the second decay time constant varies with both voltage and concentration (Connor, Levy & Parsons, 1980).

Two additional reports have recently appeared concerning the action of other ganglionic blocking drugs on sympathetic and parasympathetic preparations (Selyanko, Derkach & Skok, 1981; Rang, 1982). Selyanko *et al.*, (1981) reported an alteration in e.p.s.c. characteristics similar in many respects to those observed with procaine in sympathetic ganglia exposed to tubocurarine and hexamethonium. These authors concluded that the tubocurarine block has both 'competitive' and 'noncompetitive' components whereas hexamethonium appears to block primarily by a voltage-dependent interaction with the synaptic channels. Synaptic currents recorded in parasympathetic cells appear to be more complex than in sympathetic cells (Rang, 1981). Consequently, a comparison of the pattern of blocking actions in the two preparations is limited. However, as a generalization, hexamethonium appears to interact primarily with the ionic channel thus interfering with ion flow, whereas tubocurarine binds both at receptor

sites and within the open channel. The latter effect becomes more pronounced at negative voltages. Our results suggest that procaine may share actions of both tubocurarine and hexamethonium with the primary mode of action dependent on the membrane voltage.

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References

ADAMS, P.R. (1976). Drug blockade of open end-plate channels. *J. Physiol.*, **260,** 531–552.

ADAMS, P.R. (1977). Voltage jump analysis of procaine action at frog end-plate. *J. Physiol.*, **268,** 291–318.

AUERBACH, A. & BETZ, W. (1971). Does curare affect transmitter release? *J. Physiol.*, **213,** 691–705.

BLACKMAN, J.G., GINSBORG, B.L. & RAY, C. (1963). On the quantal release of the transmitter at a sympathetic synapse. *J. Physiol.*, **167,** 402–415.

COLOMO, F. & RAHAMIMOFF, R. (1968). Interaction between sodium and calcium ions in the process of transmitter release at the neuromuscular junction. *J. Physiol.*, **198,** 203–218.

CONNOR, E.A., LEVY, S.M. & PARSONS, R.L. (1980). Quantitative analysis of the alteration of fast excitatory postsynaptic current decay by atropine. *Neuroscience Abstracts*, **5,** 738.

DEGUCHI, T. & NARAHASHI, T. (1971). Effects of procaine on ionic conductances of end-plate membrane. *J. Pharmac. exp. Ther.*, **176,** 423–433.

DEL CASTILLO, J. & ENGBAEK, L. (1954). The nature of the neuromuscular block produced by magnesium. *J. Physiol.*, **124,** 370–384.

DEL CASTILLO, J. & KATZ, B. (1954). Quantal components of the end-plate potential. *J. Physiol.*, **124,** 560–573.

FURUKAWA, T. (1957). Properties of the procaine end-plate potential. *Jap. J. Physiol.*, **7,** 199–212.

KATZ, B. & MILEDI, R. (1975). The effect of procaine on the action of acetylcholine at the neuromuscular junction. *J. Physiol.*, **249,** 269–284.

KOBLIN, D.D. & LESTER, H.A. (1979). Voltage-dependent and voltage-independent blockade of acetylcholine receptors by local anesthetics in *Electrophorus* electroplaques. *Molec. Pharmac.*, **15,** 559–580.

KORDAS, M. (1970). The effect of procaine on neuromuscular transmission. *J. Physiol.*, **209,** 689–699.

KUBA, K. & NISHI, S. (1979). Characteristics of fast excitatory postsynaptic current in bullfrog sympathetic ganglion cells. Effects of membrane potential, temperature, and Ca ions. *Pflugers Arch. Eur. J. Physiol.*, **378,** 205–212.

MACDERMOTT, A.B., CONNOR, E.A., DIONNE, V.E. & PARSONS, R.L. (1980). Voltage clamp study of fast excitatory synaptic currents in bullfrog sympathetic ganglion cells. *J. gen. Physiol.*, **75,** 39–60.

MAENO, T. (1966). Analysis of sodium and potassium conductances in the procaine end-plate potential. *J. Physiol.*, **183,** 592–606.

MARTIN, A.R. (1966). Quantal nature of synaptic transmission. *Physiol. Rev.*, **46,** 51–66.

MARTY, A. (1978). Noise and relaxation studies of acetylcholine induced currents in the presence of procaine. *J. Physiol.*, **278,** 237–250.

RANG, H.P. (1981). The characteristics of synaptic currents and responses to acetylcholine of rat submandibular ganglion cells. *J. Physiol.*, **311,** 23–55.

RANG, H.P. (1982). The action of ganglionic blocking drugs on the synaptic responses of rat submandibular ganglion cells. *Br. J. Pharmac.*, **75,** 151–168.

RUFF, R.L. (1976). Local anesthetic alteration of miniature end-plate current fluctuations, *Biophys. J.*, **16,** 433–439.

RUFF, R.L. (1977). A quantitative analysis of local anesthetic alteration of miniature end-plate currents and end-plate current fluctuations *J. Physiol.* **264,** 89–124.

SELYANKO, A.A., DERKACH V.A. & SKOK, V.I. (1981). Effects of some ganglion blocking agents on fast excitatory postsynaptic currents in mammalian sympathetic ganglion neurons. In *Advances in Physiological Sciences: Physiology of Excitable Membranes.* ed. Salauki, J. New York: Pergamon Press. pp. 329–342.

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