SYNAPTIC TRANSMISSION IN SQUID GIANT SYNAPSE AFTER POTASSIUM CONDUCTANCE BLOCKAGE WITH EXTERNAL 3- AND 4-AMINOPYRIDINE

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The release of transmitter substance from the presynaptic terminal of the squid giant synapse, in the absence of action potentials, has been among the most useful experimental paradigms in the study of chemical synaptic transmission (Bloedel et al., 1966; Katz and Miledi, 1967; Kusano et al., 1967). Early experiments using this technique demonstrated that neither sodium nor potassium currents were necessary for synaptic transmission. More recent experiments indicate that an inward calcium current is responsible for triggering the depolarization-secretion sequence (Katz and Miledi, 1969; Llinás et al., 1972; Miledi, 1973; Llinás and Nicholson, 1975). In fact, Katz and Miledi (1969) demonstrated that following tetrodotoxin (TTX) and tetraethylammonium (TEA) administration, a presynaptic calcium-dependent spike may be generated, reliably, if the extracellular calcium concentration ($[Ca^{++}]_o$) is increased from 10 mM to 40 mM.

However, a nagging problem which has reduced the utility of this preparation is the technical difficulty inherent in the TEA administration. In the squid, TEA blocks the voltage-dependent potassium conductance (g_{K^+}) from the internal membrane surface exclusively (Armstrong and Binstock, 1965). For this reason TEA must be electrophoretically injected into the presynaptic terminal for periods as long as 4 h to achieve the required concentration. This rather laborious procedure often causes irreversible damage to the synapse leading to transmission failure.

We would like to report here that externally applied 3-aminopyridine or 4-aminopyridine (3-AmP, 4-AmP) substantially diminished g_{K^+} in the presynaptic terminal of the squid giant synapse without blocking synaptic transmission (Fig. 1). These drugs thus provide a simple tool for the study of voltage-dependent calcium conductance changes ($g_{Ca^{2+}}$) in this terminal (Llinás et al., 1975).

4-AmP has been demonstrated recently to block g_{K^+} in cockroach giant axon (Pelhate and Pichon, 1974) and 2-, 3-, and 4-AmP to block g_{K^+} in the squid giant axon (Yeh et al., 1976). The g_{K^+} blockage in the squid giant axon is, however, (*a*) not complete for transmembrane potentials larger than a few millivolts positive inside, above

which level g_{K^+} gradually reappears, and (*b*) is relieved by frequent depolarization (Yeh et al., 1976). Since similar findings were obtained in the presynaptic terminal, we increased the interval between current pulses to the pre-fiber to at least 10 s in order to avoid blockage relief and to obtain reproducible results in transmitter release. Our results have been obtained with 1, 5, and 30 mM 3- or 4-AmP in artificial seawater (cf. Llinás et al., 1972). The action of these drugs is rather fast; it generally takes between 5 and 10 min to produce a significant blockage of potassium conductance, inferred from changes in the shape of the action potential.

The synaptic transmission properties in the squid synapse, before, and after 10 min in 5 mM 3-AmP are shown in Fig. 1 A and B. Since in most cases, 3-AmP alone caused repetitive firing of the pre- and postsynaptic fibers (due to a small depolarization), 2 μM TTX was usually added to the bathing solution to prevent deterioration of the

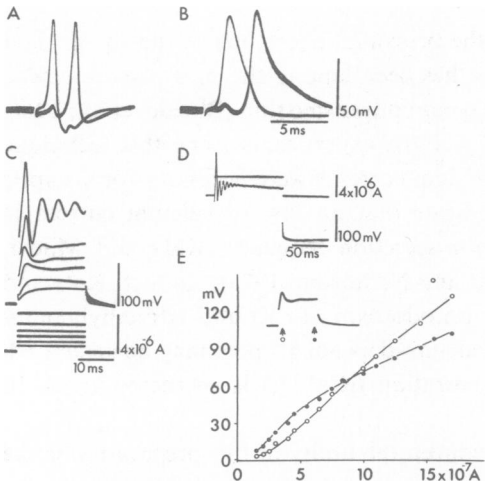

FIGURE 1

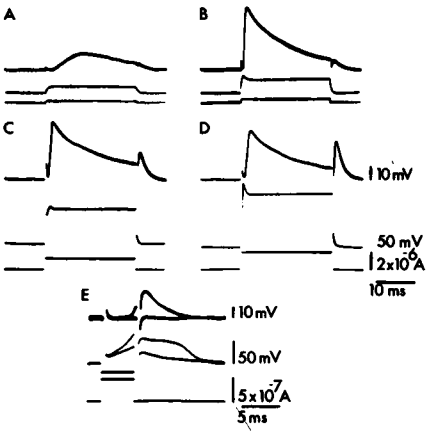

FIGURE 2

FIGURE 1 Action of 3-AmP on pre- and postsynaptic elements in the stellate ganglion. (*A*) Synaptic transmission recorded in artificial seawater. (*B*) 10 min after the addition of 5 mM 3-AmP. (*C*) Superimposed presynaptic depolarizations (upper traces) produced by current pulses of different amplitudes (lower traces) after addition of 3-AmP and TTX. Note that beyond +70 mV from resting potential there is clear activation of g_{K^+} shortly after the onset of the membrane depolarization. In some cases with depolarizations beyond +170 mV the membrane potential oscillates during the steady injection of depolarizing current, as shown in the top trace in *C* and in *D*. The graph in *E* illustrates the voltage generated at two times (arrows in insert) following the start of the presynaptic current pulse. Voltage and time calibration as indicated. Note different voltage and time calibration for *C* and *D*.

FIGURE 2 Synaptic transmitter release in the presence of 3-AmP and TTX. *A–D* are potentials generated by presynaptic current injections of increasing amplitude. Top trace: postsynaptic response; middle: presynaptic potential; bottom: presynaptic current. Note that in records *C* and *D* increased current injection produced a decrease in the amount of transmitter released and secondary transmitter output at the current break. *E* is synaptic transmission after 3-AmP and TTX in 40 mM $[Ca^{2+}]_o$. Under these circumstances, presynaptic depolarization is capable of generating calcium spikes which are accompanied by transmitter release.

synapse. This limitation of 3-AmP must be taken into consideration when studying depolarization release-coupling properties.

The decrease of g_{K^+} in the presynaptic fiber after 15 min of exposure to 3-AmP and TTX is shown in Fig. 1 C. At internal polarization levels beyond +10 mV, g_{K^+} reappears in the form of a delayed rectification. This is illustrated in Fig. 1 E. The membrane potential change produced by current pulses across the presynaptic fiber is plotted, at two different time intervals (arrows), against the amplitude of the current pulses. Beyond 64 mV the return of the delayed rectification is indicated by the difference between the voltage at the start (open circles) and near the end (filled circles) of the current pulse. Note also that at depolarization levels above +100 mV inside, a membrane potential oscillation occurs, which tends to be dampened in about 30 ms (Fig. 1 D). The actual mechanism for this phenomenon is unclear at the present. Since very high level depolarization is required to evoke this oscillation, it is doubtful that it reflects normal physiological properties of the membrane.

Synaptic transmission produced by presynaptic depolarization after 3-AmP and TTX administration is shown in Fig. 2. Very much as following TEA injection, prolonged synaptic depolarization causes sustained transmitter release (Fig. 2 A–D). At low levels of presynaptic depolarization the postsynaptic potential rises slowly and is maintained for the duration of the current pulse (Fig. 2 A). As the current is increased, the rate of rise of the postsynaptic potential increases to a peak and decays rapidly to a new level (Fig. 2 B). These records are in every way similar to those obtained following TTX and TEA administration (Katz and Miledi, 1967; Kusano et al., 1967; Llinás and Nicholson, 1975). The transmitter released, as reflected by the postsynaptic potential, is not markedly changed for periods up to 2 h after addition of 3-AmP.

In none of the cases studied could we produce a sufficiently large depolarization presynaptically to block transmitter release. That is, the "suppression potential" was not reached (Katz and Miledi, 1967; Kusano et al., 1967; Llinás and Nicholson, 1975). However, we were able to produce enough presynaptic depolarization to show a clear reduction of the on-response which was accompanied by off-release (Fig. 2 C and D), implying depolarization approaching the calcium equilibrium potential in the presynaptic terminal (Llinás and Nicholson, 1975).

After TTX, 3-AmP and 40 mM $[Ca^{2+}]_o$, typical calcium spikes were observed in the presynaptic terminal. These all-or-none voltage changes were accompanied by release of transmitter, as indicated from the postsynaptic response which they evoked (Fig. 2 E).

We conclude, therefore, that 3- and 4-AmP can substitute for TEA as a g_{K^+} blocking agent in a certain class of experiments concerning the depolarization release-coupling system in the giant synapse. Since these compounds do not appear to block $g_{Ca^{2+}}$ (at least at the concentrations studied here), they should be most useful in determining electrophysiological and pharmacological properties of the calcium current which triggers transmitter release from the presynaptic terminal. It must be stressed, however, that the 3-AmP blockage of K^+ is voltage and time dependent and, thus, that

experiments dealing with large presynaptic depolarization or with facilitation may not be feasible using this drug.

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