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MODIFICATION OF TRANSMITTER RELEASE BY IONS WHICH PROLONG THE PRESYNAPTIC ACTION POTENTIAL

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SUMMARY

1. The action of ions which increase the nerve spike duration at Ranvier's node has been studied at the neuromuscular junction of the frog.

The duration of the presynaptic action potential is increased by UO_2, Ni, Zn and TEA ions.

2. The release of transmitter after a nerve impulse is delayed and prolonged in the presence of the four ions, but the amount of evoked transmitter release estimated from the mean quantal content of e.p.p. is increased only by UO_2^{2+} and TEA. Both Ni^{2+} and Zn^{2+} decrease it.

3. The frequency of m.e.p.p.s is increased by UO_2^{2+} and decreased by Ni^{2+} and Zn^{2+} at KCl-depolarized junctions. It is not affected by TEA.

4. It has been concluded that the increased presynaptic spike duration is responsible for the delayed and prolonged transmitter release in the presence of ions which increase the nerve spike duration at Ranvier's node.

It is demonstrated that some 'specific' effects possibly resulting from ionic competition for presynaptic sites between UO_2^{2+}, Ni^{2+} or Zn^{2+} and other ions present in the Ringer may increase, interfere with, or hide the increased transmitter release that may be predicted from the lengthened presynaptic depolarization.

INTRODUCTION

One of the interesting problems of transmission at chemical synapses is the relation between the invasion of the presynaptic nerve terminal by the action potential and the transmitter release. Since the work of del Castillo & Katz (1954*c*) it is generally accepted that presynaptic depolarization increases the spontaneous release of transmitter as may be interpreted from the increase in frequency of the miniature end-plate potentials (m.e.p.p.s). Moreover, recent results obtained by Katz & Miledi (1967*b*) using the focal depolarizing pulse technique, have shown that the amount and the time course of transmitter release were affected by both the

amplitude and the duration of a presynaptic electrotonic depolarization. In particular, lengthening of presynaptic depolarization increases the following factors: (*a*) the number of quanta of transmitter released, (*b*) the minimum synaptic delay, (*c*) the time during which the probability of transmitter release is raised after a nerve impulse. Similar results could be obtained from the squid giant synapse (Katz & Miledi, 1967*c*). The question arises whether the same effect can be obtained by altering the presynaptic spike duration. This problem has been previously approached by shortening the presynaptic spike by hyperpolarizing pulses (Katz & Miledi, 1967*a*). These authors were able to suppress transmitter release by modifying the presynaptic spike by an hyperpolarizing pulse applied just after the peak of the negative phase of the presynaptic spike extracellularly recorded.

In this paper we have tried to analyse if some cations known to lengthen the spike duration at the Ranvier's node affect the transmitter release. The four ions selected have been UO_2^{2+} (Mambrini & Benoit, 1968), Ni^{2+} (Spyropoulos & Brady, 1959), Zn^{2+}, tetraethylammonium (TEA) (Schmidt & Stämpfli, 1966) and will be referred to as 'increasing nerve spike duration ions'. These ions have the common property of producing a characteristic plateau of depolarization during the falling phase of the spike at Ranvier's node. The action of these ions on synaptic transmission have been determined by studying their effects on (*a*) the time course of the presynaptic spike, (*b*) the modification of the time course of transmitter release, (*c*) the modification of the amount of transmitter release and (*d*) the effects of these ions on spontaneous transmitter release.

The results of our experiments show that increasing nerve spike duration ions increase the presynaptic spike duration in the nerve terminal. As a consequence, the time course of transmitter release is delayed and lengthened and the amount of transmitter released by nerve impulses is diversely affected. This diversity may be interpreted as due to particular effects of some of these ions on the transmitter release which interfere with the consequences of their expected effects on the time course of the presynaptic action potential.

METHODS

All experiments were performed *in vitro* at room temperature (20° C) on myoneural junctions of the sartorius muscle of the frog (*Rana esculenta*). The muscle was stretched on the convex bottom of an Altuglas bath (15 ml.) filled with Ringer solution continuously flowing. The nerve was placed on silver electrodes in a second chamber near the muscle and was stimulated supramaximally at 2/sec for the evoked transmitter release experiments. Superficial myoneural junctions were optically localized by following the myelinated nerve branches which innervate the muscular fibres of the edge of the muscle.

The normal Ringer solution contained: NaCl 112 mM; KCl 2 mM; $CaCl_2$ 2 mM; $NaHCO_3$ 2·4 mM.

Blockade of transmission was obtained by adding D-tubocurarine (1×10^{-6} g/ml.) to the physiological solution or by lowering Ca^{2+} and raising Mg^{2+} concentrations. In some experiments (when measuring the synaptic delay) Mg^{2+} replaced all the Ca^{2+} and the transmitter release was locally restored by iontophoretic application of Ca^{2+} from a $CaCl_2$ (1 M) solution filled micropipette (Katz & Miledi, 1965*c*).

Chloride salts of UO_2, Ni, Zn and TEA were perfused to the preparation by adding them to the bathing solution. The following concentrations were used: UO_2^{2+} 0·2 mM; Ni^{2+} 0·5 mM; Zn^{2+} 0·1 and 0·2 mM; TEA 0·1–0·5 mM. Replacements of solutions were achieved in about 5 min and stable responses generally obtained within 10 min. Recordings were obtained 15 min after the beginning of the solution change.

Intracellular recording of evoked or spontaneous end-plate potentials was performed by using glass micro-electrodes filled with a KCl (3 M) solution, with tip resistances of around 4–8 MΩ.

Extracellular recording techniques were used to study the action of increasing nerve spike duration ions on the time course and duration of the presynaptic action potential, and on the synaptic delay. For this purpose, glass pipettes with tips of few microns diameter, filled with a NaCl solution (0·5 M) were used. By this technique differentiated membrane potential transients could be recorded (Katz & Miledi, 1965*a*, 1967*a*) that is, the end-plate current preceded by the first or the second derivative (according to electrode location on nerve terminal) of the presynaptic action potential. The localization of the tip of the electrode on the nerve terminal is very critical and must be approached by successive trials. Automatic averaging of one or several hundred successive signals was used to overcome the low signal/noise ratio of the presynaptic spike recordings. In this case an a.c. coupled preamplifier with a long time constant was used.

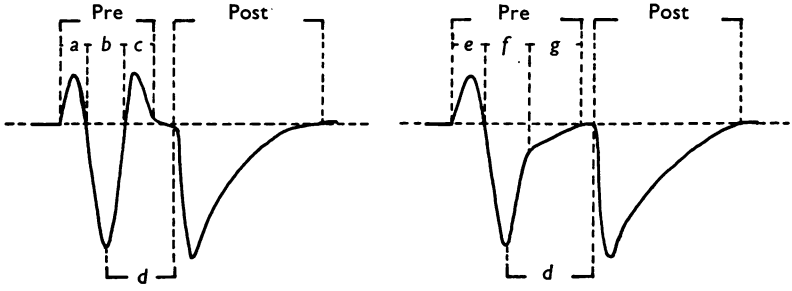

Fig. 1. Schematic representation of external recordings at an active spot of the neuromuscular junction of the frog. Horizontal brackets show the delimitation of the different phases of the second derivative of the presynaptic action potential (Pre: *a*, *b*, *c* and *e*, *f*, *g*), and the synaptic delay (*d*). Pre: presynaptic response; Post: post-synaptic response. On left: low Ca^{2+}–Mg^{2+} Ringer; on right: low Ca^{2+}–Mg^{2+} Ringer with increasing nerve spike duration ions.

Quantitative measurements. The variations of the duration of the presynaptic action potential was studied by measuring the duration of the different phases of its second derivative obtained by external focal recording. In these experiments the tip of the recording electrode was approximately positioned in the middle portion of the nerve terminal. The different phases of the second derivative of the presynaptic action potential (Fig. 1) are indicated by different letters for easier expression of the results (see Results).

The synaptic delay was measured (Katz & Miledi, 1965*b*) from external records, as the time elapsed between the maximum of the negative phase of the second derivative of the presynaptic action potential and the beginning of the post-synaptic end-plate current.

The mean quantal content m of the end-plate potential (e.p.p.) was determined at junctions blocked by low Ca^{2+} and high Mg^{2+} Ringer (del Castillo & Katz, 1954*b*) from the relation

$$m = \ln \frac{\text{number of stimuli}}{\text{number of failures}}.$$

Miniature end-plate potential (m.e.p.p.) frequency was used as an index of the spontaneous release of transmitter both at normal and KCl depolarized junctions.

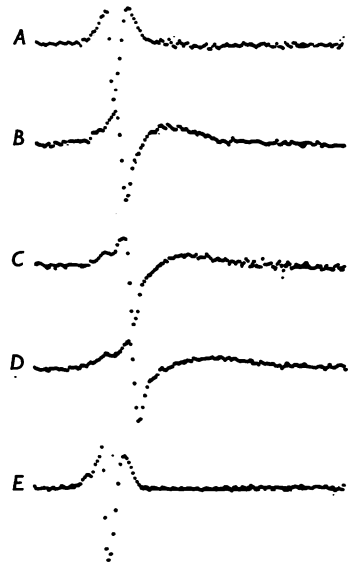

Fig. 2. Progressive modification of the presynaptic spike time course during the onset of UO_2 action. Focal recording by extracellular micro-electrode. Transmission blocked by substituting Mg^{2+} (2 mM) for Ca^{2+} in Ringer solution. Automatically averaged responses to 500 nerve impulses (each dot corresponds to 40 μsec). *A*: Ringer; *B*: Ringer + UO_2^{2+} (5 min); *C*: 15 min; *D*: 20 min; *E*: control.

RESULTS

Modification of the presynaptic spike time course and duration by increasing nerve spike duration ions

The main effects caused by ions which increase nerve spike duration on the time course of the presynaptic spike are the following:

(i) the first positive wave (*a* in Fig. 1) and the beginning of the negative wave (*b* in Fig. 1) were little or not affected (Figs. 2 and 3);

(ii) the end of the negative wave (*g* in Fig. 1) was markedly lengthened;

(iii) the second positive wave (*c* in Fig. 1) became generally undetectable (Figs. 3 and 4).

However, it was possible to observe, as in Fig. 2, both gradual reduction of amplitude and increased duration of the second positive wave during the action of the studied ions. The decrease of the amplitude of the second positive wave by ions which prolong the nerve spike duration prevented any accurate measurement of the true duration of the presynaptic action potential. Thus quantitative measurements of duration could only be made on homologous detectable phases, i.e. the first positive wave and the negative one. These results are shown in Table 1.

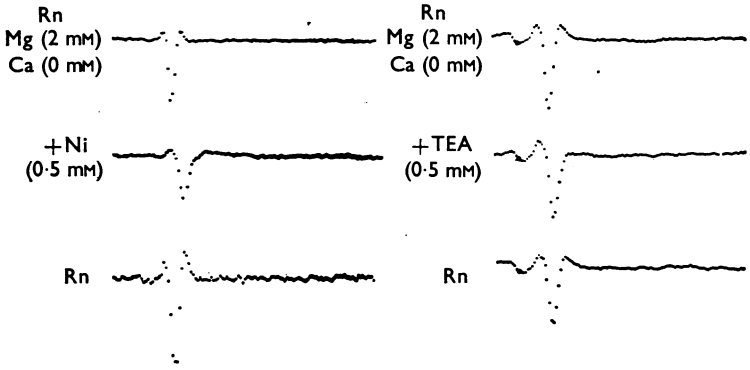

Fig. 3. Modification of the presynaptic spike time course by Ni^{2+} (left) and TEA (right). Same experimental conditions as in Fig. 2. Rn: Ringer.

TABLE 1. Modification of the time course of the second derivative of the presynaptic action potential by ions which increase nerve spike duration at Ranvier's node. The letters *a*, *b*, *c*, *e*, *f*, *g* indicate the different phases of the second derivative of the presynaptic action potential defined in Fig. 1. Durations expressed in μsec

	Ringer			Ringer + UO_2^{2+} (0·2 mM)		
	a	*b*	*c*	*e*	*f*	*g*
Average ± S.E.	309 ± 11	277 ± 20	294 ± 15	436 ± 47	416 ± 42	872 ± 100
	Ringer			Ringer + Zn^{2+} (0·1 mM)		
	a	*b*	*c*	*e*	*f*	*g*
Average ± S.E.	286 ± 31	366 ± 31	340 ± 22	275 ± 41	406 ± 40	310 ± 41
	Ringer			Ringer + TEA		
	a	*b*	*c*	*e*	*f*	*g*
Average ± S.E.	240 ± 16	430 ± 30	400 ± 69	260 ± 11	440 ± 46	290 ± 52

Modification of the minimum synaptic delay by increasing nerve spike duration ions

The minimum synaptic delay was measured on external focal recordings. Transmission was completely blocked by substituting Mg^{2+} for Ca^{2+} in Ringer solution and transmitter release restored by local iontophoretic application of Ca^{2+} (see Methods). The current through the $CaCl_2$ filled pipette was adjusted to have essentially monoquantal epps. In these experimental conditions, the minimum synaptic delay values obtained were about 500 μsec; these values were similar to those obtained by Katz & Miledi (1965*b*).

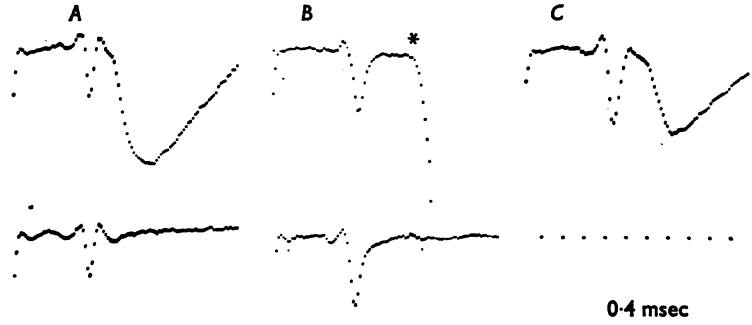

Fig. 4. Modification of the presynaptic spike time course and of the synaptic delay by UO_2^{2+} (0·2 mM). Automatically averaged responses to 100 nerve impulses. Transmission was blocked by substituting Mg^{2+} (2 mM) for Ca^{2+} in Ringer solution. In the upper records transmitter release was restored locally by iontophoretic calcium application: in lower records transmitter release was suppressed by reducing the current through the $CaCl_2$ filled pipette. All records at the same active spot. *A*: Ringer (upper and lower traces); *B*: Ringer + UO_2^{2+} (star indicates the beginning of the post-synaptic response); *C*: control.

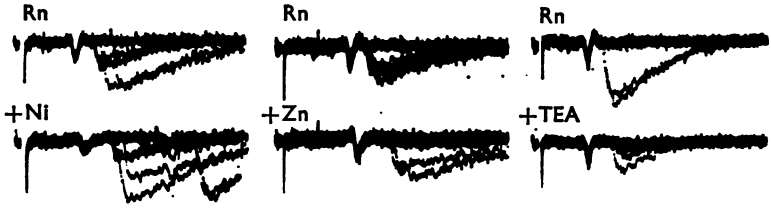

Fig. 5. Modification of synaptic delay by Ni, Zn and TEA. Same experimental conditions as in Fig. 4. In presence of the increasing nerve spike duration ions, local Ca^{2+} concentration was modified in order to keep the mean quantal content of e.p.p. constant. Upper traces: recordings in Ringer (Rn); lower traces: recordings in presence of the ions studied. Note repetitive responses to nerve stimulation in presence of Ni (left) and decreased amplitude of unit responses in presence of TEA (right).

In presence of the ions which increase nerve spike duration the minimum synaptic delay was always lengthened as shown in Figs. 4 and 5. The mean increase of the minimum synaptic delay was 170 % in the presence of UO_2^{2+} (Fig. 4), 23 % with Ni^{2+} (Fig. 5), 55 % with Zn^{2+} (Fig. 5). On the other hand, the minimum synaptic delay was increased by 21 % in TEA 0·2 mM, 29 % in 0·4 mM and 77 % in 0·5 mM (Fig. 5).

Modification of transmitter release

Katz & Miledi (1965*b*) have shown that the histogram of synaptic delays established from a junction blocked by a low Ca^{2+} high Mg^{2+} Ringer in order to have only monoquantal release (about 70 % failure to transmission) also illustrates the time course of the increased probability of transmitter release after the invasion of the presynaptic terminal by an action potential.

The action of the studied ions on the time course of transmitter release

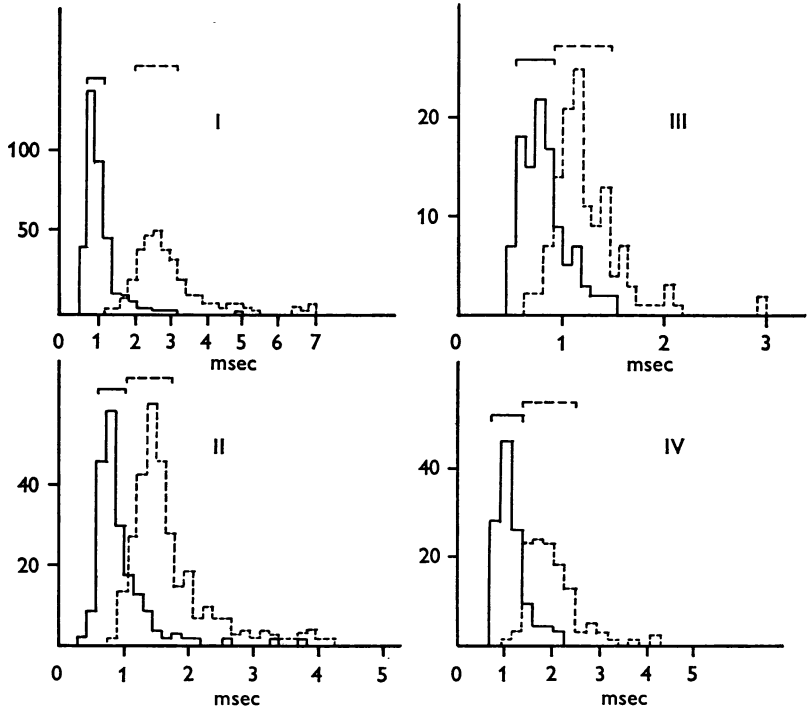

Fig. 6. Modification of synaptic delays distribution in presence of increasing nerve spike duration ions. Continuous line: Ringer histograms; interrupted line: Ringer + increasing nerve spike duration ions histograms. I: effect of UO_2^{2+} (0·2 mM); II: Ni^{2+} (0·5 mM); III: Zn^{2+} (0·2 mM); IV: TEA (0·4 mM). Horizontal brackets indicate the limits in which 70 % of the responses are included.

was studied by comparing the histograms of the synaptic delays measured in the presence of these ions to the histograms established in the control Ringer. The values of the synaptic delay were measured on records obtained during series of 500 stimulations at the same active spot of a myoneural junction. The mean quantal value had to be readjusted when the studied ions were applied because of their effects on the amount of transmitter released; this was easily done by changing the local Ca^{2+} concentration by modifying the current through the $CaCl_2$ pipette (see Methods).

In these experiments some difficulties arose from secondary phenomenon such as repetitive nerve responses to nerve stimulation in presence of Ni^{2+} (Fig. 5), or curare-like effects of TEA (Fig. 5) on the post-synaptic membrane (Koketsu, 1958). These effects have limited the range of the concentrations for the studied ions used.

Some of the histograms obtained under these experimental conditions are shown in Fig. 6. The main effects observed in presence of ions which increase the nerve spike duration were: an increased minimum latency and increased temporal dispersion of measurements. This last effect is clearly shown by the increase in the time interval in which 70% of the measurements are included (Fig. 6, horizontal brackets).

Modification of the amount of transmitter released

When myoneural transmission was blocked by curare, the amplitude of evoked e.p.p.s could be increased by UO_2^{2+} (Mambrini & Benoit, 1968) and by TEA (Koketsu, 1958) or decreased by Ni^{2+} (Miledi, 1966; Mambrini & Benoit, 1967) and Zn^{2+}.

Since variations of e.p.p. amplitude at curarized junction in presence of the studied ions might be due to pre or post-synaptic effects or both, the modification of the mean quantal content of e.p.p. (m) by these ions has been studied at the myoneural junctions blocked by Ringer solution containing low Ca^{2+} (0·4 mM) and high Mg^{2+} (5 mM) (see Methods). These modifications have been expressed by the ratio R:

$$R = \frac{m' \text{ (in presence of studied ions)}}{m \text{ (in presence of control solution)}}.$$

The following averaged values have been obtained for R: UO_2^{2+}: $R = 18$; TEA (0·2 mM): $R = 3{\cdot}6$; Ni^{2+}: $R = 0{\cdot}94$, Zn^{2+}: $R = 0{\cdot}44$ (see Table 2). During prolonged washing with control Ringer solution after Zn^{2+} action an irreversible increase of m was generally observed (see Table 2).

Effects on spontaneous release of transmitter

In order to clarify the diversity of the effects of the studied ions on the amount of evoked transmitter released, further experiments were made to study the modification of the frequency of m.e.p.p.s at resting and KCl depolarized junctions by ions which increase nerve spike duration. It was previously observed that the miniature frequency at resting junctions was

TABLE 2. Modification of mean quantal content (m) of e.p.p. by UO_2^{2+}, Ni^{2+} and Zn^{2+} (R is defined in the text)

Ringer	Ringer + UO_2^{2+}	Ringer	R
0·37	2·30	0·40	5·97
0·67	3·75	0·50	6·41
0·79	5·40	0·59	7·82
1·01	7·50	1·12	7·04
0·72	6·5	0·67	9·35
0·22	2·66	0·10	16·62
0·50	10	0·72	16·60
0·82	3·9	0·56	5·49
0·015	1·03	0·01	69
0·11	2·46	0·11	22
0·08	1·72	0·025	32·75
			Average ± S.E. 18 ± 5·7

Ringer	Ringer + Ni^{2+}	Ringer	R
3·20	0·20	3·20	0·06
4·60	0·15	4·60	0·03
4·60	0·08	3·20	0·02
3·20	0·15	2·10	0·05
2·10	0·10	1·75	0·05
1·60	0·03	1·50	0·02
1·25	0·03	1·50	0·02
4·60	0·35	3·20	0·09
2·80	0·16	2·30	0·06
			Average ± S.E. 0·04 ± 0·008

Ringer	Ringer + Zn^{2+}	R	Ringer (7 min)	(20 min)	(35 min)
0·90	0·27	0·30	0·80	0·80	1·55
1·16	0·60	0·51	0·20	2·13	3·90
0·60	0·15	0·25	0·85	0·80	—
0·50	0·38	0·76	0·25	—	—
0·50	0·25	0·50	0·75	1·16	1·41
0·80	0·35	0·43	1·45	1·45	1·55
		Average ± S.E. 0·44 ± 0·07			

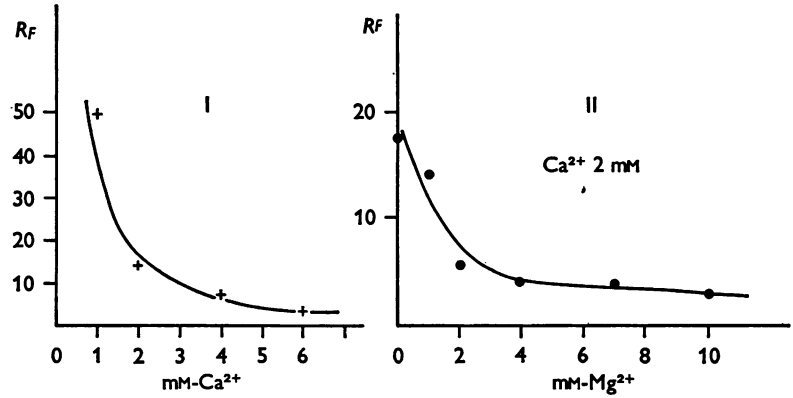

Fig. 7. Modification of the frequency of m.e.p.p.s by UO_2^{2+} for different Ca^{2+} (I) and Mg^{2+} (II) concentrations in Ringer solution. Ordinates: R_F = ratio of the frequency of m.e.p.p.s in Ringer + UO_2^{2+}/the frequency of m.e.p.p.s in Ringer. R_F was measured for each Ca^{2+} or Mg^{2+} concentration. Abscissae: Ca^{2+} or Mg^{2+} concentration. For curve II, Ca^{2+} (2 mM) was present through all experiment. All points obtained at the same end-plate.

TABLE 3. Effect of UO_2^{2+} on e.p.p. miniatures frequency for different Na^+ concentrations in Ringer

$$N = \frac{\text{m.e.p.p. frequency in Ringer} + UO_2^{2+}\ (0{\cdot}2\ \text{mM})}{\text{m.e.p.p. frequency in Ringer}}.$$

N was measured at each junction in presence of Ringer containing 112 mM-NaCl (normal concentration) and then in Ringer containing 67 mM-NaCl (60 % of normal concentration). m.e.p.p. frequencies are expressed in number of m.e.p.p. min^{-1}

		Rn	Rn + UO_2^{2+}	Rn	N	$\frac{N(\text{Na } 100\,\%)}{N(\text{Na } 60\,\%)}$
Ca 2 mM	Na 100 %	56	856	52	15·8	1·38
	Na 60 %	98	988	75	11·4	
	Na 100 %	54	1200	54	22	1·57
	Na 60 %	98	1148	66	14	
	Na 100 %	24	478	25	19·5	1·95
	Na 60 %	32	300	27	10	
Ca 4 mM	Na 100 %	101	816	132	7	1·40
	Na 60 %	109	560	110	5	
	Na 100 %	84	359	84	4·22	1·93
	Na 60 %	122	306	122	2·35	
	Na 100 %	23	180	22	7·99	1·05
	Na 60 %	35	253	32	7·55	
Ca 2 mM	Na 100 %	90	924	68	11·5	1·23
Mg 4 mM	Na 60 %	47	485	57	9·32	
						Average ± S.E.
						1·50 ± 0·12

increased 17 times by the addition of UO_2^{2+} to a normal Ringer solution (Mambrini & Benoit, 1968). The cation concentration of the Ringer influences this effect of UO_2^{2+}. Thus, increases in Ca^{2+} and Mg^{2+} concentration depress the effect of UO_2^{2+} on m.e.p.p.s frequency (Fig. 7) as does removal of Na^+ from the Ringer at any given Ca^{2+} concentration (Table 3).

When neuromuscular junctions are depolarized by adding KCl (10 mM) to normal Ringer, the m.e.p.p.s frequency is increased (Liley, 1956). In such condition, the addition of UO_2^{2+} to Ringer increased the m.e.p.p.s frequency to such an extent that the m.e.p.p.s could no longer be counted.

The other studied ions have different effects on m.e.p.p.s frequency. It was previously shown that Ni^{2+} does not significantly affect the m.e.p.p.s frequency at resting junctions but depresses m.e.p.p.s frequency by 53 % at depolarized junction (Mambrini & Benoit, 1967).

On the other hand, m.e.p.p.s frequency was not significantly affected 10 min after the addition of Zn^{2+} (0·1 or 0·2 mM) to the Ringer solution. A prolongation of this application for longer periods brought out an irreversible increase of the frequency of m.e.p.p.s, which did not disappear even after a prolonged washing with normal Ringer solution. At KCl-depolarized junctions, after a transient decrease of m.e.p.p.s frequency, Zn^{2+} caused again a consistent and irreversible increase of m.e.p.p.s frequency.

TEA (0·3 mM) did not affect the frequency of m.e.p.p.s but the mean amplitude was reduced by up to 60 % in presence of TEA. This curare-like effect of TEA made experiments in KCl-depolarized junctions difficult. However, in some experiments TEA was added to a Ringer containing 10 mM-KCl, and no apparent change of m.e.p.p.s frequency was observed.

DISCUSSION

Action of studied ions on the presynaptic action potential. The present results strongly suggest that the duration of the presynaptic action potential in the nerve terminal was lengthened by the ions which prolong the spike duration at Ranvier's node. This increase in duration occurred without important modification of both the first positive wave and the beginning of the negative one and mainly consisted in the development of a slow phase at the end of the negative wave of the extracellular records. It must be emphasized that the main negative phase of the focal spike coincides with the ascent and peak of the membrane potential change. Then the lengthening of the end of the negative wave of the second derivative of the presynaptic action potential may therefore be interpreted as a slowing down of the repolarizing phase of the action potential in nerve terminal. The question arises now if this slowing down is or is not accompanied by a plateau similar to the one observed at Ranvier's node, charac-

terized by a delayed rapid phase of repolarization when UO_2^{2+} is added to the Ringer (Mambrini & Benoit, 1968). If such a rapid phase did occur at the end of the presynaptic action potential, one would expect that a late wave would appear on the second derivative of the presynaptic spike. Such a late wave was never observed in our experiments. Moreover, during the action of the studied ions, a progressive delayed second positive wave was never observed. The only features recorded consistently were the progressive lengthening and the reduction in amplitude of this second

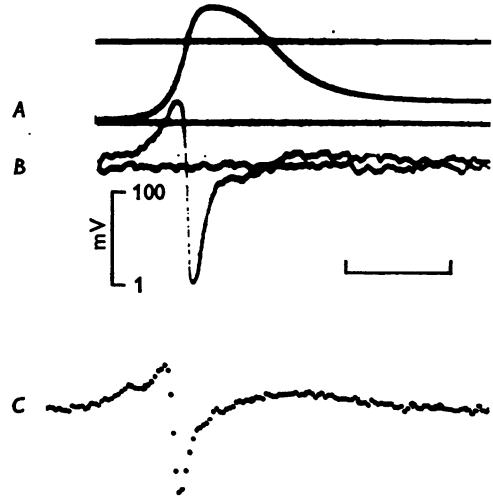

Fig. 8. Comparison between external recordings of muscle and nerve terminal action potentials. *A*: intracellular recording of muscle action potential; *B*: simultaneous extracellular recording of the second derivative of muscular action potential at same fibre (*A* and *B* from Katz & Miledi 1965*a*); *C*: extracellular recording of nerve terminal action potential in presence of UO_2^{2+} (0·2 mM). Calibration for *A* and *B*: 100 mV for intracellular recording, 1 mV for extracellular recording; 1 msec for *A* and *B*. Calibration for *C*: each dot corresponds to 40 μsec.

positive wave. It is possible then to conclude that the time course of the presynaptic action potential in the nerve terminals under the action of the ions which increase the spike duration at Ranvier's node would be probably similar to the time course of a normal muscle action potential. Such an interpretation finds a good support in Fig. 8 where the second derivative of a presynaptic action potential in presence of UO_2^{2+} is compared with the second derivative of a muscular action potential recorded by Katz & Miledi (1965*a*).

Action on the time course of transmitter release. The present experiments also show that the studied ions both increase the minimum latency of transmitter release and prolong the period of time in which transmitter is

released from the nerve terminal after the arrival of nerve impulse. These results are consistent with the observed lengthening of the presynaptic spike in presence of these ions, since Katz & Miledi (1967*b*) showed at tetrodotoxin poisoned junctions that increasing the duration of an electrotonic depolarization of the nerve terminal increases both the minimum synaptic delay and the time during which the probabilty of transmitter release is increased after a depolarizing pulse.

Effect on the amount of transmitter release. If the preceding results are similar for the different studied ions, it is not the same when we consider their action on the amount of transmitter released by a nerve impulse. UO_2^{2+} and TEA increase the mean quantal content of e.p.p. but Ni^{2+} and Zn^{2+} decrease it. Since such results with Ni^{2+} and Zn^{2+} could be interpreted as showing that the amount of transmitter release is independent of the duration of the presynaptic spike, a series of experiments were performed to determine if the studied ions were able to affect the amount of transmitter released by another way than by altering the duration of the presynaptic depolarization.

Experiments on KCl depolarized neuromuscular junctions show that UO_2^{2+} increase the m.e.p.p.s frequency whereas Ni^{2+} and Zn^{2+} decrease it and it may be concluded from these experiments that UO_2^{2+}, Ni^{2+} and Zn^{2+} may have a specific effect on transmitter release. Little decrease in m.e.p.p.s frequency was observed after adding TEA to Ringer. This may be interpreted as a loss of some m.e.p.p.s in the base line noise, due to the curare-like post-synaptic effect of TEA. Therefore, among the four ions we have studied, TEA may be considered as the only one which is probably deprived of specific effects on transmitter release, and the increase in mean quantal content caused by TEA must be interpreted as due to a lengthening of the presynaptic spike duration. For the three other ions, it is interesting to emphasize that they are divalent cations and thus probably able to compete with the divalent cations of the Ringer for same membrane sites. Such a competition can be accepted for Ni^{2+} and probably Zn^{2+}, since it was previously demonstrated (Mambrini & Benoit, 1969) that Ni^{2+} may cause a shift of the straight line relating the logarithm of extracellular Ca^{2+} concentration and the logarithm of mean quantal content (Rahamimoff & Colomo, 1967), without change in the slope of the line. Ni^{2+} can therefore be considered as acting like Mg^{2+}, competing then with Ca^{2+} for presynaptic sites. Such a competition between Ni^{2+} and Ca^{2+} was already proposed by Meves (1963) for the case of Ranvier's node membrane. A specific effect of UO_2^{2+} on transmitter release is demonstrated by the increased spontaneous transmitter release observed in presence of this ion. Such a specific effect reinforces the already shown effects of UO_2^{2+} on presynaptic depolarization, which increases evoked trans-

mitter release. This specific effect could be attributed to a depolarizing action of UO_2^{2+} on the presynaptic ending, but such an action seems unlikely since it was rarely observed at Ranvier's node (Mambrini & Benoit, 1968). Moreover, the effect of UO_2^{2+} on transmitter release is visible even at KCl depolarized junctions. A second hypothesis may be that uranyl acts at the presynaptic membrane by competing with some ions playing a rôle in transmitter release. This is suggested by the fact that the action of UO_2^{2+} on spontaneous transmitter release may be antagonized by Na^+ deprivation or by Ca^{2+} or Mg^{2+} addition. Now since a competition appears to exist between Na^+ and Ca^{2+} for specific sites of the presynaptic membrane (Kelly, 1965; Gage & Quastel, 1965, 1966; Rahamimoff & Colomo, 1967; Colomo & Rahamimoff, 1968), the specific effect of UO_2^{2+} could be interpreted as due to an interference of UO_2^{2+} in the competition between Ca^{2+} and Na^+ on presynaptic sites. The preceding interpretation may also be supported by the observation that UO_2^{2+} does not affect the slope of the line relating the logarithm of Ca^{2+} concentration and the logarithm of mean quantal content of e.p.p. (Mambrini & Benoit, 1969).

An alternative possibility would be that UO_2^{2+} is able to act at some step of the transmitter release mechanism in a way similar to Ca^{2+}. Its action would be then limited to a competition with the divalent cation of the milieu for the same presynaptic sites. This last possibility is difficult to accept since Ca^{2+} must be present to allow evoked transmitter release (see Fig. 4).

The opposition between specific effects of UO_2^{2+} and those of Ni^{2+} or Zn^{2+} on transmitter release could be related to differences between the ligands which these ions are able to bind with (Takahashi, Murai & Sasaki, 1960; Takahashi, Usuda & Ehara, 1962; Sandow & Isaacson, 1966; Metuzals, 1969) but more evidence is necessary to confirm this.

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