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Dantrolene and the effect of temperature on the spontaneous release of transmitter at the frog neuromuscular junction

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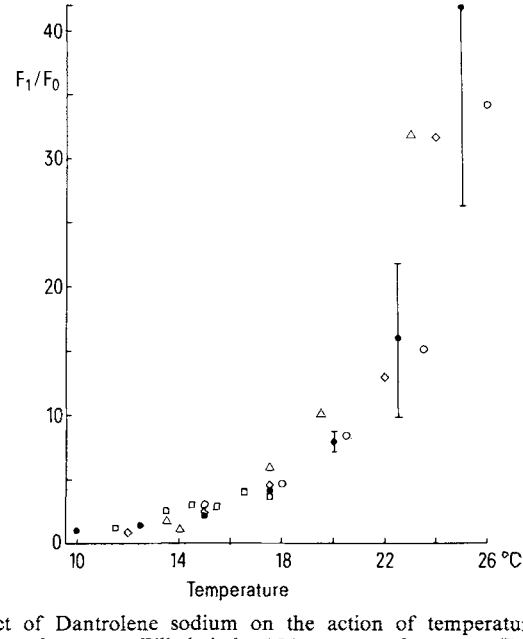
Summary. The characteristic effect of temperature on m.e.p.p. frequency at the amphibian neuromuscular junction is unaltered by the presence of Dantrolene (an agent that is believed to reduce the efflux of Ca^{2+} from intracellular stores) or by changes in $[Ca^{2+}]_o$. It is concluded that temperature affects the release system directly, with a transition temperature at about 16 °C.

The action of a number of agents on the spontaneous release of transmitter (as measured by the frequency of miniature endplate potentials, m.e.p.p.s) at the amphibian and mammalian neuromuscular junction are explicable in terms of altered concentration of intracellular free calcium ($[Ca^{2+}]_i$) at the presynaptic terminals. Experimental results suggest that raising $[Ca^{2+}]_i$ by accelerating Ca^{2+} influx or by releasing Ca^{2+} from intracellular storage sites causes a rise in m.e.p.p. frequency[2,3]. M.e.p.p. frequency at the frog neuromuscular junction is also particularly sensitive to raised temperature, especially above 15 °C where a high Q_{10} and activation energy were recorded[3,4]. The same temperature sensitivity was found in low extracellular Ca^{2+} ($[Ca^{2+}]_o$ buffered at 5×10^{-7} M) and it was therefore suggested that it was possible that the main effect of raised temperature (apart from increasing the activity of the Ca^{2+}-pumps which would serve to lower $[Ca^{2+}]_i$) might be to promote the release of Ca^{2+} from intracellular storage sites[3]. We have now studied the effect of temperature on spontaneous release of transmitter in the presence of Dantrolene sodium (DaNa), an agent that is believed to reduce the steady state level of $[Ca^{2+}]_i$ at amphibian presynaptic terminals by reducing Ca^{2+}-efflux from intracellular stores and which therefore depresses m.e.p.p. frequency markedly[5].

Electrophysiological recordings of m.e.p.p.s were made from the cutaneous pectoris nerve-muscle preparation of the frog *Rana pipiens* by conventional techniques[6]. Preparations were equilibrated in saline containing DaNa (10 μg ml^{-1}) for 40 min at 10 °C, and m.e.p.p. frequency fell to 30%; the effect of varying temperature in the range 10–26 °C was then determined.

The results are shown in the figure, where m.e.p.p. frequency is expressed as a ratio of the control frequency at 10 °C in presence of DaNa (so permitting direct comparison with experiments in the absence of DaNa[3]). It can be seen that DaNa does not markedly modify the characteristic effect of temperature on m.e.p.p. frequency, i.e. spontaneous release is still little affected by temperature change below 16 °C, but is highly temperature-sensitive about this point.

A single experiment was also carried out in which the effect of temperature was studied in the presence of DaNa and in which $[Ca^{2+}]_o$ was buffered at a low level of 5×10^{-7} M. Low $[Ca^{2+}]_o$ also reduces m.e.p.p. frequency, but does not alter the normal overall effect of temperature[3], and the figure shows that this finding is also true in the presence of DaNa.



Effect of Dantrolene sodium on the action of temperature on m.e.p.p. frequency. Filled circles (●) represent frequency (F_1) as a ratio of the frequency at 10 °C (F_0), $[Ca^{2+}]_o = 1.8$ mM, mean of 13 separate experiments ± SE of mean where this exceeds the diameter of the points (data from Duncan and Statham[3]). Open symbols represent results of 4 separate experiments carried out in the presence of Dantrolene sodium (10 μg ml^{-1}); $[Ca^{2+}]_o = 1.8$ mM except triangles (△), where $[Ca^{2+}]_o = 5 \times 10^{-7}$ M.

Evidence obtained previously suggests that the action of DaNa is independent of $[Ca^{2+}]_o$, that it is antagonized by theophylline and that it reduces the release of Ca^{2+} from intracellular storage sites thereby reducing the steady-state level of $[Ca^{2+}]_i$ at the frog presynaptic terminals[5]. Comparable results have been obtained with the effect of DaNa at the rat adrenal medulla[7]. Since the temperature sensitivity of spontaneous release is not affected by a reduction of $[Ca^{2+}]_o$ (Duncan and Statham[3]), nor is it modified in the presence of DaNa, which might be expected to inhibit the release of Ca^{2+} from intracellular membrane systems[5], we now conclude that the major effect of temperature is probably not to modify $[Ca^{2+}]_i$. It appears more likely that the release mechanism itself is directly and markedly modified by temperature, perhaps associated with a phase-change in the phospholipoprotein system of the plasma membrane and/or the synaptic vesicles at about 16 °C. Such a conclusion is in accord with a) the very different effects of diamide on m.e.p.p. frequency above and below a critical temperature of 16 °C which have been recently described[8] and b) with the observation that stimulation of m.e.p.p. frequency by the ionophore A23187 shows a marked temperature sensitivity in this range, the effect being virtually absent at temperatures below 17 °C (Statham and Duncan[9]). Unfortunately, it is not possible to observe the effects of ionophore treatment on the temperature-frequency curve, as plotted in this communication, since A23187 treatment results in a progressively increasing rate of spontaneous release[9].

It is now clear that there is a complex interrelationship between transmitter release (both spontaneous and evoked), temperature and $[Ca^{2+}]_i$ (Publicover and Duncan[10]); it seems that the particularly high Q_{10} and activation energy shown by the release system above 16 °C is found only under conditions of low $[Ca^{2+}]_i$. Thus, the $Q_{10}^{10\text{-}20\,°C}$ for m.e.p.p. frequency is 10, both under normal conditions and when the terminals are depolarized with 20 mM $[K^+]_o$ in the absence of extracellular Ca^{2+} (conditions in which $[Ca^{2+}]_i$ is low). However, the $Q_{10}^{10\text{-}20\,°C}$ is only 4 for evoked release (as measured by the quantal content of the e.p.p.) and for m.e.p.p. frequency in the depolarized preparation in normal extracellular Ca^{2+} (conditions in which $[Ca^{2+}]_i$ is high)[4].

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Catecholamines in bovine semen

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Summary. In seminal plasma 5×10^{-6} M noradrenaline was found to induce head-to-head association in bull spermatozoa. The sum of noradrenaline and adrenaline in freshly collected semen was 10 ng/ml (5.2×10^{-8} M), i.e., about 100 times lower than previously reported.

With the aid of a colorimetric method Brochart[1] demonstrated the presence of some substance, thought to be adrenaline (A), in semen from 3 mammalian species. In bull semen the concentration was 1.0 µg/ml (5.5×10^{-6} M). Later[2] the substance was considered to be chiefly noradrenaline (NA). The agent was shown to be present in both semen and seminal plasma.

Catecholamines elicit head-to-head association (HHA) in bull sperm washed with Tris buffered medium or Tyrode solution, whereas the oxidation product adrenochrome dissociates spermatozoa associated by washing[3]. The lowest concentrations tested were for A 2×10^{-6}, for NA 5×10^{-5} M, both substances being approximately equally efficient at 5×10^{-5} M. The fact that this effect is inhibited by α- and β-adrenergic blockers and is induced also by exogeneous cAMP seems to indicate the existence of a mechanism triggered by some related compound, to which spermatozoa are more sensitive than to the catecholamines tested. We conceive that the changes brought about in the cell surface by this mechanism, resulting in the artefact HHA, play some role in prefertilization events.

In normal semen HHA occurs only as a result of inactivation by oxidation of a substance located in the sperm surface and seminal plasma[4], preventing sperm from associating by way of another mechanism. Under natural conditions catecholamines do not induce HHA in semen, either because their concentration is too low or because this process is blocked in some way. We have therefore studied whether NA can induce HHA in bull sperm diluted with seminal plasma and have submitted samples of normal bull semen to a sensitive and specific analytical procedure, giving the sum of A and NA, calculated below as NA and symbolized by NA*.

Table 1. Induction of head-to head association in bull spermatozoa by 5×10^{-6} M noradrenaline in seminal plasma

	Association (%)	$\bar{D} \pm SD$	Deviation from zero	
			t	p
Control	0.59	1.20 ± 0.888	4.279	< 0.01
Sample	1.78			

Spermatozoa from 2 ejaculates, from different bulls. $\bar{D}$: mean difference between control and sample counts of 10 determinations; at least 500 cells counted in each determination.