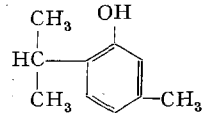
The Influence of Thymol on the Sodium Efflux in Barnacle Muscle Fibres

In a recent paper[1] from this laboratory it was shown that the response of the Na efflux in barnacle muscle fibres to caffeine is diphasic. Inhibition of the Na efflux followed by stimulation was explained by supposing that the initial rise in the internal free Ca^{2+} concentration caused inhibition of the Na^+–K^+ ATPase, and that a further rise in Ca^{2+} concentration caused stimulation of the Ca^{2+}-sensitive component of the Na efflux. The present experiments were undertaken to see whether thymol, an alkyl derivative of phenol (as indicated below) produces similar effects which could be attributed to reduced Ca^{2+} uptake or increased Ca^{2+} release by the



Thymol

sarcoplasmic reticulum (SR). Thymol was chosen mainly because there is evidence[2] that 2 mM thymol abolishes Ca^{2+} uptake by the SR without depressing the activity of the Ca^{2+}-ATPase. Doubling the concentration of thymol, however, leads to enzyme inhibition. It seemed possible therefore that experiments with thymol might shed some light on the problem of how caffeine acts and how it alters Na transport.

Single muscle fibres were dissected from specimens of the barnacle *Balanus nubilus* or *B. aquila*, cannulated and then loaded with ^{22}Na by microinjection in the same way as squid axons[3] and crab muscle fibres[4]. The method of measuring ^{22}Na in the washing-out samples and the fibres was essentially that described by BITTAR[5] and BITTAR, CALDWELL and LOWE[6]. $^{22}NaCl$ was supplied by Amersham-Searle Corp. (SKS-1). The composition of the bathing medium was the same as that given previously[7]. Thymol was obtained from Merck & Co., Inc., Rahaway, N.J. All experiments were done between 22 and 24 °C.

Earlier studies[8] showed that concentrations of ethyl alcohol in the bathing medium as high as 1% (by volume) fail to change the Na efflux significantly, and that a 0.1% concentration is without effect. Preliminary trials, first, with 10^{-3} M thymol in 1% ethanol-artificial sea water were done. As shown in Figure 1a, external application of 10^{-3} M thymol caused a dramatic rise in the Na efflux. The magnitude of this stimulation as calculated on the basis of the change in $1/[Na^*]\, d[Na^*]/dt$ averaged 1376% ($n = 2$). This result prompted us to reduce the concentration of thymol to 2.5×10^{-4} M in 0.1% ethanol-artificial sea water only to find that it produced inhibition of the Na efflux as shown in Figure 1b. The size of this inhibitory effect averaged 49% ($n = 2$), a value in good agreement with that calculated from the size of the step-down in the rate of Na efflux. This striking difference in behavior of the Na efflux toward 2.5×10^{-4} M thymol was thought to be due to a rise in the internal free Ca^{2+} concentration, leading to inhibition of the Na^+–K^+ ATPase. Hence the next step was to somewhat increase the concentration of thymol in the bathing medium. As is seen in Figure 2a, external application of 5×10^{-4} M thymol resulted in a diphasic response. Initially there was a fall in the Na efflux, and then a marked rise. The size of the inhibition averaged 51% ($n = 6$). In most instances, however, the stimulatory phase turned out to be biphasic, as shown in Figure 2b. Calculations of the size of the stimulatory effect took into account the maximum rate coefficient for Na loss, usually observed after the onset of the second stimulatory phase, and the rate coefficient for Na loss prior to application of the thymol. On this basis the magnitude of the stimulatory effect averaged 701% ($n = 6$). For the purpose of comparison, slope analysis of the log efflux plots were tried, and these gave an average value of 514%.

Since these kinetic results were similar to those obtained with caffeine, it seemed natural to wonder if stimulation involved activation of a ouabain-insensitive

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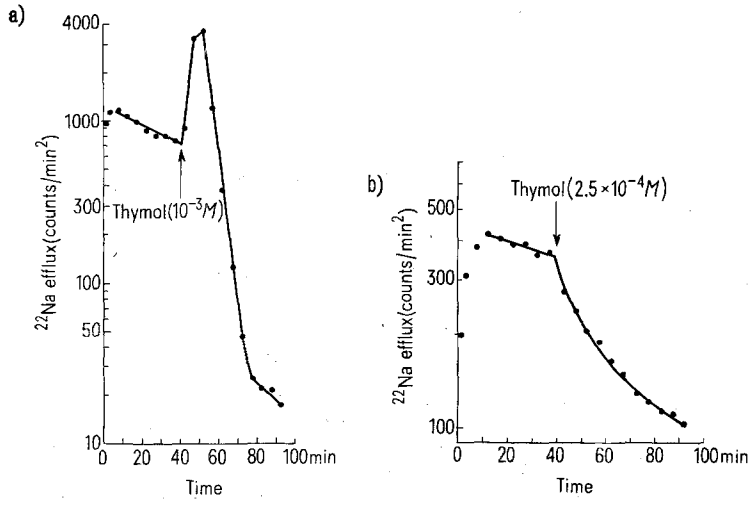

Fig. 1. a) Na efflux from a barnacle muscle fibre during treatment with $10^{-3}M$ thymol (semilog plot). b) Na efflux from a barnacle muscle fibre during treatment with $2.5\times10^{-4}M$ thymol.

component of the Na efflux. Experiments were therefore done with 10^{-4} M ouabain, followed by 5×10^{-4} M thymol. As illustrated by Figure 3, thymol caused a huge rise in the Na efflux; again, 2 definite peaks are observed. Estimates based on rate constant plots led to an average value of 363% ($n = 6$) for the size of the first peak, and 2306% ($n = 4$) for the second peak. The latter though not an over-estimate, could not be compared with the second stimulatory phase observed in fibres not treated with ouabain. By taking into account, however, the rate constant for Na loss found prior to applying ouabain rather than 30 min after its application, an average value of 1,120% was obtained. This is taken to mean that ouabain greatly increases the sensitivity of these fibres to thymol by inactivating the Na^+–K^+ ATPase. Another salient feature of the experiments carried out with ouabain is the rapid onset of the stimulatory effect. This is also true when caffeine is applied to ouabain-treated fibres[9]. It thus appears that, on the one hand, thymol (or caffeine) has the ability to reduce the Na efflux, presumably because it raises the free Ca^{2+} concentration and hence, the [Ca ATP]$^{-2}$ concentration. This, in turn, leads to suppression of the Na^+–K^+ ATPase. On the other hand, thymol also has the ability to stimulate the Na efflux by raising the internal free Ca^{2+} to levels which greatly activate the ouabain-insensitive Na efflux.

These considerations lead to the idea that for the case of barnacle muscle fibres it is feasible to study cell injury and how the SR may behave as a pacemaker of plasma membrane activity. That thymol, moreover, can be used as a contractile agent is shown by the fact that a 5×10^{-4} M concentration shortened barnacle fibres to at least $^1/_3$ their original length. It is worth noting that SAKAI, FUJII and SHIMUZU[10] and JANKE, OBERDISSE and PETZOLDT[11] had found thymol to produce 'rapid cooling contracture' and to augment KCl-induced contracture in amphibian sartorius muscle.

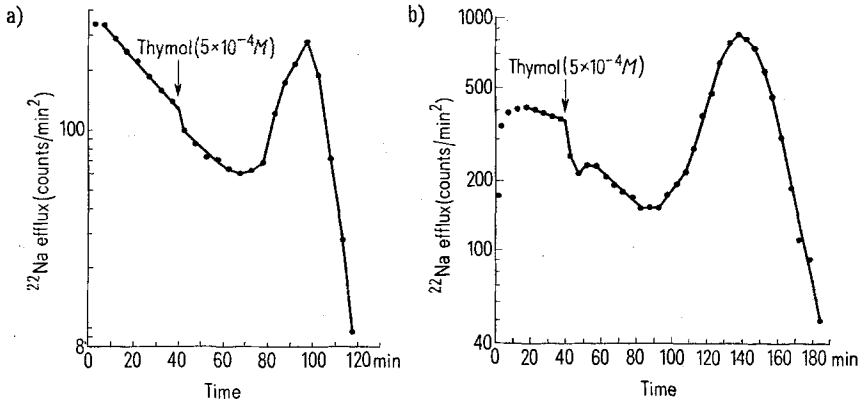

Fig. 2. a) Diphasic response of the Na efflux to $5\times10^{-4}M$ thymol. b) To illustrate not only the diphasic response of the Na efflux to $5\times10^{-4}M$ thymol but also the 2 stimulatory peaks.

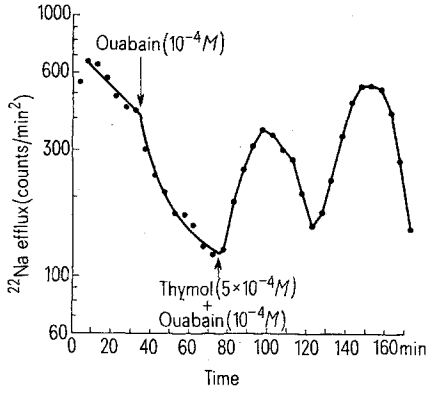

Fig. 3. The response of the ouabain-insensitive Na efflux to $5\times10^{-4}M$ thymol.

Lastly, one may well ask, what is the significance of the two-stimulatory peaks? If we are to accept the idea that the peaks are caused by a rising free Ca^{2+} concentration in the sarcoplasm, one could then explain the first peak as being due to interruption by thymol of Ca^{2+} uptake by the SR, and the second peak as being due to increased release of 'bound' Ca^{2+}. Experiments designed to account for this two-peak phenomenon are now in progress[12].

Zusammenfassung. Nachweis, dass Thymol sowie Coffein in der Barnakel-Muskelfaser eine primäre Hemmung mit nachfolgender Stimulierung des Natrium-Auswärtsstromes hervorruft.

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[12] Acknowledgment. This work was supported in part by grants from the Wisconsin Heart Association, the Office of Naval Research, and the National Science Foundation. DONNA BROWN is a Postdoctoral Fellow of the Wisconsin Heart Association.