The mechanism of Ca\(^{2+}\) release from the SR of permeabilised guinea-pig and rat ureteric smooth muscle

Th.V. Burdyga, M.J. Taggart, C. Crichton, G.L. Smith, Susan Wray

"Physiological Laboratory, University of Liverpool, Crown Street, Liverpool, L69 3BX, UK"
"Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow, G12 8QQ, UK"

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Abstract

Recent work has indicated that there is a major difference in the Ca\(^{2+}\) store of smooth muscle from rat and guinea-pig ureter; with the rat store being agonist-sensitive but ryanodine insensitive and the guinea-pig store being ryanodine sensitive but agonist insensitive [Th.V. Burdyga, M.J. Taggart, S. Wray, J. Physiol. 489 (1995) 327–335]. We have therefore examined directly the mechanism of Ca\(^{2+}\) release from the internal Ca\(^{2+}\) store (SR). Following permeabilisation with \(\alpha\)-toxin or \(\beta\)-escin the SR was Ca\(^{2+}\)-loaded before application of carbachol or caffeine. Only carbachol evoked a transient contraction in rat ureter. The carbachol-induced contraction was blocked by heparin and cyclopiazonic acid (CPA) but not ryanodine. Only caffeine produced contraction in guinea-pig ureter, and this was blocked by ryanodine. Direct application of IP\(_3\) caused a small transient contraction in rat but not guinea-pig ureter. We conclude that rat ureter possesses only an IP\(_3\) sensitive store while guinea-pig ureter only has a ryanodine sensitive store.

Keywords: Calcium ion; Ureter; Ryanodine

1. Introduction

The internal Ca\(^{2+}\) store (sarcoplasmic reticulum, SR) of smooth muscles is not homogeneous. The relative amounts of Ca\(^{2+}\) released by the IP\(_3\)-sensitive part (IP\(_3\)-induced-calcium release, IICR) and the Ca\(^{2+}\)-sensitive part (calcium-induced calcium release, CICR) differ between smooth muscles [1,2]. It has recently been found that differences in the mechanism of SR Ca\(^{2+}\) release also occur within the same smooth muscle from different species [3]. Thus in the ureter of the guinea-pig, Ca\(^{2+}\) release from the SR was caffeine and ryanodine sensitive but agonist insensitive, while in the ureter of the rat it was caffeine and ryanodine insensitive but agonist sensitive [3]. On the basis of these observations it has been postulated that in the guinea-pig ureter the store Ca\(^{2+}\) release is mediated by the ryanodine receptor on the CICR channels, while in the rat ureter it is mediated by IP\(_3\) receptor on the IICR channels. Such extreme differences in the same smooth muscle are interesting and may lead to functional differences between the rat and guinea-pig ureters. The above study however was carried out on intact ureteric smooth muscle and therefore it was not possible to obtain more direct evidence as to the nature and the properties of the intracellular Ca\(^{2+}\) stores. For example it is not possi-
ble to directly apply IP₃ or its inhibitor, heparin, in intact preparations [4].

Permeabilisation of smooth muscle sarcolemma has proved to be a valuable technique for investigation of the nature and the properties of the SR, as membrane impermeant substances can be used and the intracellular [Ca²⁺] ([Ca²⁺]), around the myofilaments and SR can be precisely controlled [5–7]. Furthermore if the smooth muscle is permeabilised using α-toxin or β-escin, then cell surface receptors are left intact and thus the muscle retains its responsiveness to agonists and pharmacomechanical coupling can be studied [7]. Thus in the present experiments we have used these agents to permeabilise strips of smooth muscle from ureters to elucidate the nature of the Ca²⁺ release mechanism from the SR in guinea-pigs and rats. We demonstrate that there are direct differences in the nature of the Ca²⁺ store they possess.

2. Methods

Adult rats or guinea-pigs were killed by a blow to the head and exsanguination and the ureters quickly removed. Small strips (200 μm wide, 100 μm thick and 1 mm long), cleared of fat, were attached to a force transducer (Akers AE17625) and at a fixed point with snares in a small tissue bath. The method of α-toxin permeabilisation was as previously described [8,9]. Briefly, before permeabilisation the strip was contracted with high K⁺ to confirm viability and provide a reference contraction. The strip was then placed in a mock intracellular solution (solution A, Table 1) with a [Ca²⁺] of 100 μM which caused only a transient contraction in intact preparations and after 10–20 min in this solution the tension had declined virtually to baseline. Following addition of α-toxin to solution A there was a slow elevation of tension as Ca²⁺ gained access to the myofilaments; which reached a plateau after 10 min. (The toxin was then removed, with little change in tension.) Subsequent lowering of the [Ca²⁺] to approximately 1 nM (solution B) caused a rapid relaxation of force. The permeabilised strip was then incubated in 250 nM Ca²⁺ (0.2 mM EGTA, solution C) to allow SR accumulation of Ca²⁺. The addition of carbachol (100 μM, for ~1 min) caused a transient contraction. Non-permeabilised strips in such mock-intracellular solution never responded with contraction to carbachol application. This response, along with the gradual elevation of tension during α-toxin permeabilisation were taken as indicative of successful permeabilisation. β-escin was used in the same way in some preparations to allow access of larger molecular weight substances (<30,000) to the intercellular space e.g., heparin [7]. The permeabilisation of the rat ureter was much easier to achieve than the guinea-pig ureter. More reliable results were obtained with β-escin skinning than α-toxin for the guinea-pig ureter. Successful permeabilisation in the guinea-pig was judged by the increase in tension during permeabilisation and response to caffeine (10 mM). It can be seen from Fig. 1 that both preparations responded to Ca²⁺-mobilizing agents with transient contractions. Provided that interval between applications was kept constant (normally 5 min), highly reproducible responses could be repeatedly obtained. Following permeabilisation the experiments were carried out at room temperature. Solution compositions are given in Table 1, including the normal Tyrodes (D) used for storing muscles in before permeabilisation, and are also detailed elsewhere [8,9]. All chemicals were purchased from Sigma except, α-toxin (Glasgow

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Solution composition (in mmol l⁻¹ except where stated)</th>
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<tbody>
<tr>
<td>Solution</td>
<td>A</td>
</tr>
<tr>
<td>K⁺</td>
<td>120</td>
</tr>
<tr>
<td>Na⁺</td>
<td>40</td>
</tr>
<tr>
<td>Total Mg²⁺</td>
<td>7.0</td>
</tr>
<tr>
<td>Total Ca²⁺</td>
<td>10.06</td>
</tr>
<tr>
<td>Free Ca²⁺ (μM)</td>
<td>100</td>
</tr>
<tr>
<td>EGTA</td>
<td>10</td>
</tr>
<tr>
<td>CH₃SO₃⁻</td>
<td>100</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>14</td>
</tr>
<tr>
<td>ATP</td>
<td>5</td>
</tr>
<tr>
<td>CrP</td>
<td>15</td>
</tr>
<tr>
<td>HEPES</td>
<td>25</td>
</tr>
<tr>
<td>Pi</td>
<td>1</td>
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The pH solutions A, B and C was 7.2; free Mg²⁺ was 2.3 mM; solution D had a pH of 7.4. Experiments were carried out at room temperature (20–22°C).

Pi = inorganic phosphate.
Fig. 1. (A) The contractile responses of permeabilised guinea-pig (Ai) and rat (Aii) ureteric smooth muscle to caffeine (10 mM) and carbachol (100 μM). (B) The effect of ryanodine on the response of the guinea-pig ureter to caffeine (Bi), and the rat ureter to carbachol (Bii). All experiments were performed in 250 nM Ca\(^{2+}\) and at room temperature. The small artifacts on this and subsequent records are due to air bubbles.

University, and IP\(_3\) and heparin (Calbiochem). \(n\), is the number of preparations.

3. Results

3.1. Effects of caffeine and carbachol on the permeabilised ureter of the guinea-pig and rat

Permeabilised strips of ureter of both species were exposed to applications of 10 mM caffeine or 100 μM carbachol in the presence of 250 nM Ca\(^{2+}\) (0.2 mM EGTA) to identify the mechanism mediating Ca\(^{2+}\) release in the two species. Fig. 1 shows that, as in intact preparations [3] guinea-pig ureter responded to caffeine but did not respond to carbachol (Fig. 1i), while the rat ureter responded to carbachol but did not respond to caffeine (Fig. 1ii, typical of three others).

3.2. Effects of ryanodine

Fig. 1B illustrates the effect of ryanodine on caffeine and carbachol contractions of the ureter. Ryanodine eventually, effectively blocked caffeine responses of the guinea-pig ureter, \(n = 2\) (Fig. 1Bi), but had little or no effects on the carbachol contractions of the rat ureter, \(n = 4\) (Fig. 1Bii). Thus Ca\(^{2+}\) release in the guinea-pig ureter is caffeine and ryanodine sensitive.

3.3. Effect of cyclopiazonic acid

Cyclopiazonic acid (CPA) completely blocks Mg\(^{2+}\)–ATP dependent Ca\(^{2+}\) accumulation by the sarcoplasmic reticulum (SR) of the ureter [10]. In intact preparations, CPA inhibited both carbachol- and caffeine-induced contractions in the rat and guinea-pig ureter, respectively [3] indicating that they possess a similar mechanism for Ca\(^{2+}\) uptake into the store. In the permeabilised preparations, CPA also abolished the carbachol contractions of the rat ureter (Fig. 2A, \(n = 4\)), confirming its ability to block Ca\(^{2+}\) accumulation by the SR in this tissue.

3.4. Comparison of the effects of carbachol, IP\(_3\) and heparin

The next step in these experiments was to identify the mechanism of Ca\(^{2+}\) release from the SR elicited
Fig. 2. (A) The effect of cyclopiazonic acid on the contractile response to carbachol in the permeabilised rat ureter. (B) The response of the permeabilised rat ureter to carbachol and IP₃ (100 μM). (C) The effect of heparin on the contractile response of rat ureter, permeabilised with β-escin, to carbachol.

Fig. 3. The effect of carbachol on Ca²⁺-activated force in permeabilised rat ureter, at maximally activating (100 μM) and low (0.3 μM) [Ca²⁺]. It can be seen that at the high [Ca²⁺] there is a small potentiation of the tonic component of force, which is essentially absent at the sub-maximal [Ca²⁺].
by carbachol in the rat ureter. Muscarinic activation of smooth muscles is reported to involve the production of IP$_3$, which then acts to release Ca$^{2+}$ from the store [11]. We therefore compared the effects of carbachol and IP$_3$ on contraction. Fig. 2B shows that IP$_3$ (100 $\mu$M) can directly produce a contraction although its amplitude was smaller ($18 \pm 2\% \ n = 4$) than that elicited by carbachol. If IP$_3$ is involved in the Ca$^{2+}$ release from the store then it should be antagonised by heparin [4,7]. The effects of heparin on the carbachol contraction of the rat ureter were therefore also studied. Fig. 2C illustrates the effect of cumulative additions of heparin at 50 and 100 $\mu$M on the carbachol contraction of a $\beta$-escin permeabilised preparation. It was found that heparin at 100 $\mu$M produced strong, although not complete, suppression of carbachol contraction of the rat ureter. Thus it is concluded that IP$_3$ mediates the effects of carbachol in the rat ureter.

3.5. Does carbachol modulate the contractile response induced by Ca$^{2+}$?

In accordance with a number of observations, pharmaco-mechanical coupling consists not only of activation of Ca$^{2+}$ release from the store, but also potentiation of the contractile response to Ca$^{2+}$ i.e., sensitisation [11]. In the next series of experiments therefore the effects of carbachol on Ca$^{2+}$-activated force were studied. Force was produced by maximal (100 $\mu$M) and submaximal (0.3–0.7 $\mu$M) [Ca$^{2+}$]. It was found that when [Ca$^{2+}$] was raised to 100 $\mu$M the force generated was clearly biphasic showing the transient, phasic and sustained tonic components of force. Addition of carbachol potentiated the tonic component of the contractile response generated by this high concentration of Ca$^{2+}$ by 12–22% (Fig. 3). With a submaximal concentration of Ca the phasic component of force was normally missing and the sensitising effect of carbachol on force was small (<10%) (Fig. 3), and difficult to detect at these low levels of force production.

4. Discussion

The data obtained in this paper show, for the first time, that ureteric smooth muscle can be successfully permeabilised. Both $\alpha$-toxin and $\beta$-escin are suitable agents, with the latter being better for the guinea-pig ureteric preparations. The results obtained in the permeabilised preparations add further, and more direct evidence, to support the conclusion that the same smooth muscle, the ureter, from two species, can have very different mechanisms mediating the release of Ca$^{2+}$ from the SR.

The lack of response to caffeine in rat ureteric smooth muscle and to carbachol in guinea-pig ureteric smooth muscle, was highly consistent between preparations of the two species. This is in agreement with the data obtained from intact preparations [3]. This suggests, exclusively IICR and CICR release mechanisms, respectively. This is also supported by the ryanodine data, which showed blockage of the guinea-pig ureteric response to caffeine but had no effect on the response to carbachol in the rat ureter. Clearly, however, the stores are similar in other respects e.g., transient release to appropriate stimuli and blocking of Ca$^{2+}$ uptake by CPA.

The transient response of the rat ureter to direct application of IP$_3$ was consistently less than the response seen to carbachol. One explanation for this discrepancy is the known rapid hydrolysis of exogenously applied IP$_3$ to permeabilised preparations, as permeabilised smooth muscle has a high endogenous IP$_3$-phosphatase activity [12]. This also leads to difficulties in knowing the concentration of IP$_3$ seen by the SR. Further support for carbachol eliciting its response via IP$_3$ was provided by the heparin data. Heparin was used as an antagonist of IP$_3$ on the SR and its presence led to a dose-related decrease in the response of the ureter to carbachol. Thus taking these data together, it seems reasonable to conclude that in the rat ureter, carbachol leads to an elevation of IP$_3$ which causes Ca$^{2+}$ release from the SR and hence contraction. This mechanism was absent from guinea-pig ureter, even though in intact guinea-pig ureter carbachol does elicit a small increase in tension due to an increase in transarcolemmal Ca$^{2+}$ influx [3].

As noted already it is generally accepted that the internal Ca$^{2+}$ store is stimulated to release Ca$^{2+}$ by IICR and CICR mechanisms. It is also known that the relative contribution or size of these two components of the store may vary between smooth muscles or mode of stimulation. For example, the Ca$^{2+}$ released from pulmonary smooth muscle SR by IICR and
CICR mechanisms are equal in magnitude, whereas in portal vein the ICR releasable store is greater than that of the CICR store [1]. The only other smooth muscle previously demonstrated to lack an IP₃-sensitive store is the intestinal longitudinal muscle from rabbits [13], whereas the myometrium of rat appears to lack CICR [14–16]. In the case of the intestinal longitudinal muscle, when IP₃ was added to permeabilised preparations, Ca²⁺ was not released [13]. In the case of the myometrium, although mRNA encoding for ryanodine receptors has been detected [14], no functional role has yet been established [15]. Furthermore caffeine does not cause a rise in Ca²⁺ or contraction ([16,17] and Taggart and Wray, unpublished observations).

To investigate whether carbachol had a sensitizing effect on the ureter, in addition to its ability to release Ca²⁺ from the SR, we applied it to a contraction produced by maximal [Ca²⁺] (100 μM). The small potentiation seen in force, indicates that this is not a very potent effect and suggests it is not as important as its ability to release Ca²⁺ from the SR. This is further supported by the very small effect of carbachol on sensitisation at lower, more physiological, [Ca²⁺] (Fig. 3).

5. Conclusion

In conclusion, the data in this paper suggests that direct application of carbachol or IP₃ cause Ca²⁺ release from the SR, and contraction of, rat but not guinea-pig ureter, whereas caffeine has these effects in guinea-pig but not rat ureter. Further work is required to clarify if these differences are due to differences in SR receptor abundance or other factors, such as the geometry of the ureteric SR, in each species. Thus in attempting to understand the role of IICR and CICR in cells it is important to realise that not all cells possess both types of stores. That such differences should exist in the same tissue but between species is also fascinating, and may lead to differences in neurohormonal modulation of force in the ureter.

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References