Functional evidence for calcium-induced calcium release in isolated rat vibrissal Merkel cell mechanoreceptors

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1. Single unit recordings were made from Merkel cell (sinus hair type I; St I) and sinus hair type II (St II) mechanoreceptors in isolated rat vibrissae. Responses were determined as the number of spikes evoked by controlled mechanical displacement of the hair shaft for 5 s every 30 s.

2. Superfusion of caffeine (10 mM) increased the responses of Merkel cell receptors by 50–180% of control (mean ± S.E.M., 64 ± 12·6%, $n = 6$, $P < 0·001$). Similar concentrations of caffeine inhibited St II receptor responses by 20–60% (mean ± S.E.M., 35 ± 8%, $n = 5$, $P < 0·01$). In both receptor types, caffeine induced a low-frequency increase in spontaneous firing.

3. When Merkel cell receptor responses were completely blocked by superfusion of high Mg^{2+}-containing solution (to competitively block Ca^{2+} influx) caffeine had no effect when added after complete inhibition, but when added during partial inhibition of responses, the Mg^{2+}-induced inhibition was transiently reversed or halted. This suggests that Ca^{2+} influx was a prerequisite for the action of caffeine.

4. Ryanodine (1 μM) increased the responses of Merkel cell receptors to mechanical stimulation by 7–60% (mean ± S.E.M., 32 ± 10·9%, $n = 5$, $P < 0·05$) but had no effect on St II receptor responses.

5. The Ca^{2+}-induced Ca^{2+} release (CICR) inhibitor procaine inhibited St I receptor responses in a concentration-dependent manner. Near-maximal inhibition was attained with 100 μM procaine. In four St I units, mean responses were depressed to 25% of control values. When both procaine (100 μM) and caffeine (10 mM) were introduced together, no net effect was seen. The responses of St II receptors were little affected by up to 100 μM procaine superfusion.

6. It is concluded that the mechano-electrical transduction process in St I receptors (but not St II) includes a CICR pathway. Taken with previous findings on the role of Merkel cells, it is likely that CICR is occurring in the Merkel cells.

Merkel cells are found in the skin and many cutaneous appendages of all mammals and some submammalian species (see Tachibana, 1995, for recent review). They make synaptiform contact with the expanded terminals arising from myelinated afferent axons. A cluster of these Merkel cell–neurite complexes make up one unit which is referred to as slowly adapting type I (SA I) in the skin (Iggo & Muir, 1969) and sinus hair type I (St I) in sinus hairs or vibrissae (Gottschaldt, Iggo & Young, 1973). Though the morphological features of Merkel cells (e.g. cytoplasmic projections, dense cored granules polarized towards the synaptiform region), are suggestive of a mechano-sensory/transducer function of Merkel cells in the complex, direct functional evidence has been lacking and the role of Merkel cells in mechanotransduction remains the subject of enduring controversy (e.g. Ikeda, Yamashita, Ono & Ogawa, 1994; Mills & Diamond, 1995; Senok, Baumann & Halata, 1996). In their natural location, Merkel cells are small, transparent and difficult to access without extensive enzymatic and mechanical treatment, by which time they have lost their characteristic shape and possibly function (Yamashita, Akaike, Wakamori, Ikeda & Ogawa, 1992).

It was recently reported that the antimalarial drug chloroquine specifically impaired the responses of Merkel cell receptors in isolated vibrissae and that chloroquine induced extensive ultrastructural changes which were largely restricted to Merkel cells while sparing the nerve endings and other cells in the hair follicle (Senok, Halata & Baumann, 1996). It has been suggested that chloroquine interferes with intracellular Ca^{2+} mobilization (Okwuasaba, Otubu & Udoh, 1990). We therefore sought to establish whether in fact pharmacological manipulation of intra-

cellular Ca^{2+} release would affect the function of Merkel cell receptors in isolated vibrissae. For comparison, the same experiments were carried out on sinus hair type II (St II) receptors, which are also slowly adapting vibrissal mechano-receptors arising from myelinated afferents of similar calibre but have no specialized cellular attachment analogous to the Merkel cell.

Our results suggest that intracellular Ca^{2+} mobilization by the process of Ca^{2+}-induced Ca^{2+} release (CICR) is involved in the sensory transduction of Merkel cell receptors. This being the first report of a possible involvement of CICR in mechano-sensory transduction, the likely role of such a pathway in Merkel cell receptor transduction is considered. A preliminary report of this work has been published in abstract form (Senok & Baumann, 1996).

METHODS

Details of the technique for isolating and recording from single vibrissae have been described elsewhere (Baumann, Chan, Halata, Senok & Yung, 1996; Senok *et al.* 1996). All experiments were carried out with the prior approval of the Animal Research Ethics Committee of the Chinese University of Hong Kong. Adult male Sprague–Dawley rats (250–300 g body weight) were initially anaesthetized with urethane (20% w/v, 6 ml kg^{-1} I.P.) and then subsequently killed by an intracardiac injection of a similar amount of urethane. A whisker pad was rapidly excised and placed in a pool of synthetic interstitial fluid (SIF; Bretag, 1969), containing (mM): Na^+, 145; K^+, 3·5; Ca^{2+}, 1·5; Mg^{2+}, 0·69; Cl^-, 114; HCO_3^-, 26·2; PO_4^{2-}, 1·7; SO_4^{2-}, 0·69; gluconate, 9·6; glucose, 5·55; and sucrose, 7·6. The solution was continuously bubbled with a gas mixture of 95% O_2–5% CO_2 to achieve a pH of 7·4. Single vibrissae were dissected from the whisker pad with a short length of the deep vibrissal nerve (DVN) still attached. The thick sinus body capsule enclosing the blood sinuses was slit longitudinally and the blood washed off.

For electrophysiological recordings, a hair follicle was mounted on a Sylgard platform in the recording chamber using fine insect pins and continuously superfused with SIF at 33–35 °C using a peristaltic pump. Fine strands were teased from the nerve and attached to a pair of silver wire electrodes while testing for mechano-sensory activity by mechanically displacing the hair shaft while monitoring spike activity on an oscilloscope and through loudspeakers. When suitable single unit responses were obtained, the hair shaft was firmly attached to the probe of a mechanical transducer about 0·5 cm from the hair bulb. Feedback-controlled displacements were applied to the hair shaft for 5 s (with 500 ms ramps and 4000 ms plateau) every 30 s. St I (Merkel cell) and St II receptors were functionally distinguished on the basis of their response characteristics (Gottschaldt *et al.* 1973; Senok & Baumann 1994; Baumann *et al.* 1996).

The drugs caffeine and ryanodine were purchased from RBI, while procaine hydrochloride was from Sigma. Test substances were prepared in normal solution and superfused by switching from the normal to the drug-containing solution for the required duration (usually 10–15 min). High-Mg^{2+}-containing SIF (2·5–5 mM) was prepared by addition of $MgCl_2$ to normal SIF.

Data were collected and digitized on-line using a general purpose laboratory interface (CED1401 plus, Cambridge Electronic Design, Cambridge, UK) and an 80486-based computer. These included the total number of spikes during the 5 s stimulus period (bin width, 1 ms), during the dynamic phase (the 500 ms rising ramp) and the static phase (time interval, 3500–4500 ms, arbitrarily chosen as a period when the receptor response to sustained mechanical displacement was considered to be most stable). Interspike interval data and spontaneous receptor activity between stimuli (bin width, 10 ms) were also monitored and recorded on-line. Response data were normalized by taking an early period of ten stimuli as 100%, to eliminate inter-unit variations. For drug treatments, the period of maximum inhibition or excitation was compared with the immediate pretreatment period. SigmaPlot and SigmaStat (Jandel Scientific) were used for mathematical transformation, graph plotting and statistical analysis. Results are expressed as the means ± standard error of the mean. A P value $< 0·05$ was considered significant (Student's t test).

RESULTS

Effect of caffeine

St I. Caffeine caused a marked increase in responses of St I receptors to mechanical stimulation (Fig. 1*A* and *B*). The effect was concentration dependent (Fig. 1*B*) and varied widely in magnitude between receptors, ranging from 50 to 180% of control values when 10 mM caffeine was superfused for 10 min (mean, 64 ± 12·6% of control, $P < 0·001$, $n = 6$; Fig. 1*D*). Caffeine also induced a transient low-frequency spontaneous discharge. In contrast to the increase in responses to mechanical stimulation, this was usually decreased (Fig. 1*B*) or had stopped completely before stopping the drug. Proportionately, responses were affected to a much greater extent during the static than the dynamic phase of stimulation (Fig. 1*B*).

St II. St II receptor responses to mechanical stimulation were inhibited by caffeine (Fig. 1*C*). Superfusion of 10 mM caffeine for 15 min resulted in a 20–60% inhibition of St II responses to mechanical stimulation (mean, 35 ± 8% of control in 5 St II receptors, Fig. 1*D*). As in the St I receptor, caffeine also caused an increase in spontaneous firing of the St II receptors (Fig. 1*C*).

Effect of caffeine on Mg^{2+}-induced inhibition of St I

To test the effect of competitively blocking Ca^{2+} influx on the observed caffeine effect, high-Mg^{2+} (2·5 or 5 mM)-containing SIF was superfused before addition of caffeine in the continued presence of high Mg^{2+}. On its own, high Mg^{2+} blocked St I responses to mechanical stimulation (Fig. 2*A*). Caffeine had no effect when added after complete inhibition by 5 mM Mg^{2+} (Fig. 2*C*), but when added during partial inhibition of responses, the Mg^{2+}-induced inhibition of the static counts was temporarily halted or reversed (Fig. 2*B*).

Effect of ryanodine

Ryanodine increased responses to mechanical stimulation in the St I receptor (Fig. 3*A*) but had no effect on St II receptor responses (Fig. 3*B*). After washout, the increase in responses showed little tendency to return to baseline. Superfusion of 10 mM caffeine led to a further rise in responses which recovered to pre-ryanodine levels (Fig. 3*A*). When treated with 10 μM ryanodine, St I responses rose

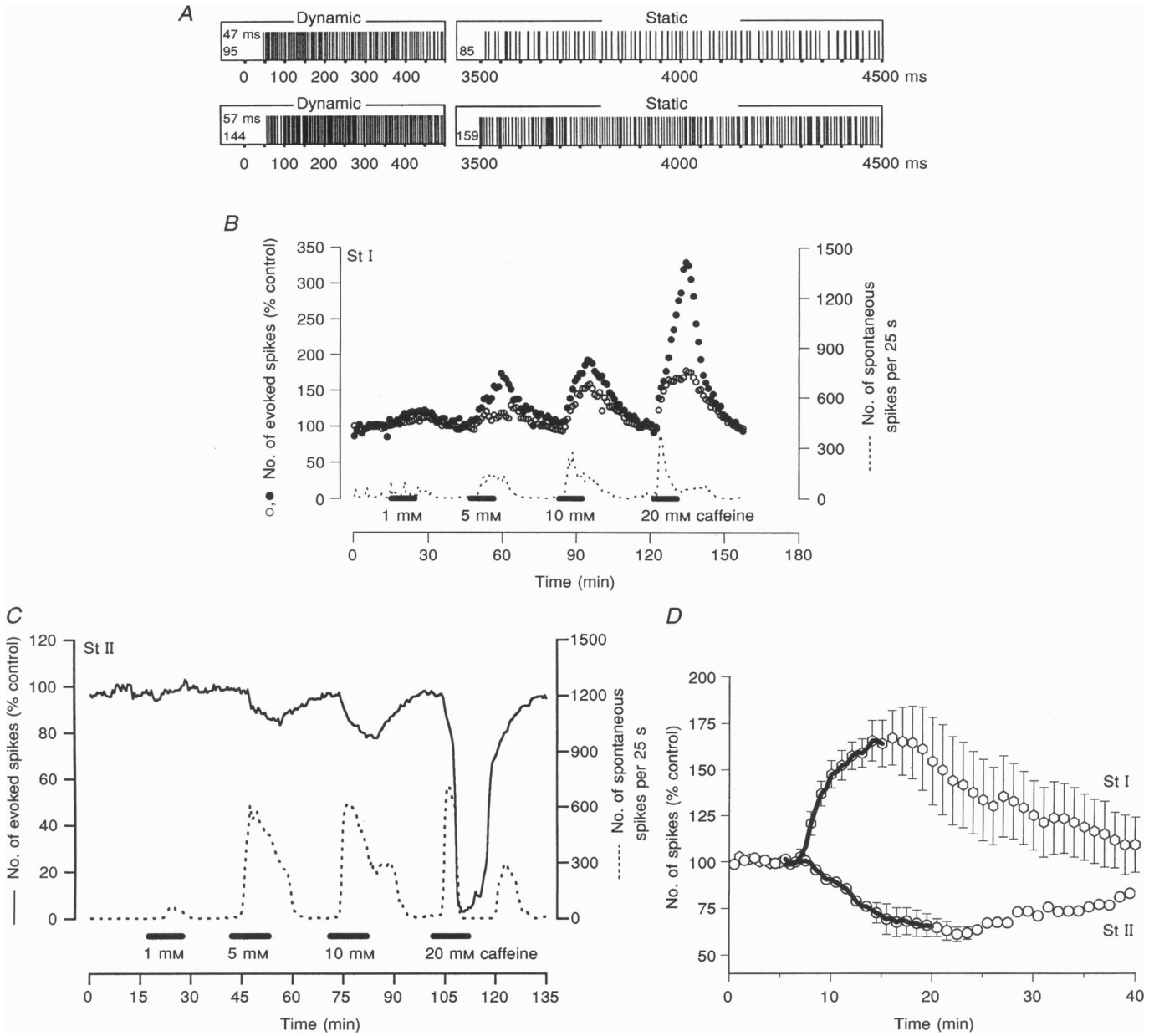

Figure 1. Effect of caffeine on the function of St I (Merkel cell) and St II receptors

Caffeine increases the responsiveness of St I receptors to repeated (5 s every 30 s) ramp-hold displacements. *A*, sample trains of transistor-to-transistor logic (TTL) pulses representing spikes before (upper panel) and at the end of (lower panel) treatment with 10 mM caffeine. The response during the 500 ms ramp phase ('dynamic') and the period 3500–4500 ms phase ('static') of a 5 s stimulus are shown. The numbers in the 'dynamic' boxes refer to the time (ms) when the first spike was recorded and the number of spikes during the 500 ms period. The numbers in the 'static' boxes represent the number of spikes during the 1000 ms static phase. Bin width for all response data is 1 ms. Spike trains were extracted off-line using stored data from the same St I receptor shown in *B*. *B*, a representative St I receptor showing responses during the dynamic (○) and static phases (●) plotted as a percentage of control (taken as the ten stimuli immediately preceding the first caffeine treatment). Caffeine increased responses in a concentration-dependent manner. Proportionately, caffeine had a greater effect on the static than the dynamic responses. The difference between the change in static and dynamic counts was significant at all four concentrations. Spontaneous spikes were also increased in a concentration-dependent manner in this receptor. The bin width of collection of spontaneous activity was 10 ms. *C*, a representative St II receptor showing the effect of caffeine on the total number of spikes during repeated stimuli. The responsiveness of St II receptors to mechanical stimulation was inhibited by caffeine. Caffeine, however, increased spontaneous firing in the St II during the 25 s intervals between stimuli. *D*, effect of 10 mM caffeine (thick line) on total counts (during 5 s stimuli) of six St I and five St II receptors. St I responses were increased by 64·0 ± 12·6% of control ($P < 0·001$) while St II receptor responses were decreased by 35·0 ± 8% of control ($P < 0·01$). Error bars are S.E.M.

initially but were often not sustained, crashing below the control levels (Fig. 3C). Though of lesser magnitude than the effect of caffeine, the effect of ryanodine (132 ± 11% of control in 5 St I receptors, inset in Fig. 1A) was significant ($P < 0.05$). Ryanodine-treated St I and St II receptors subsequently responded to caffeine treatment in a qualitatively similar manner, as described above (Fig. 3A and B).

Effect of procaine

Procaine inhibited St I receptor responses in a dose-dependent manner. Near-maximal inhibition was attained with 100 μM procaine (Fig. 4A). In four St I units, mean responses were depressed to 25% of control values (Fig. 4E). The individual effects of procaine (100 μM) and caffeine (10 mM) were not observed when they were simultaneously superfused (Fig. 4D). The responses of St II receptors were little affected by procaine superfusion; 50 μM procaine had no effect on responses while 100 μM reduced responses by 10·0 ± 3·3% of control (Fig. 4E). When present, spontaneous activity in St II receptors was silenced by procaine (Fig. 4B).

St II receptors were completely blocked by procaine at concentrations above 100 μM (Fig. 4C). Recovery was, however, rapid and rather abrupt (within 10 min, compared with 30–45 min for St I).

DISCUSSION

Evidence for Ca^{2+}-induced Ca^{2+} release

Millimolar concentrations of caffeine release Ca^{2+} from intracellular stores by opening the ryanodine receptor (RyR) intracellular release channels (for recent reviews see Sawynok & Yaksh, 1993; Pozzan, Rizzuto, Volpe & Meldolesi, 1994). It is used to activate Ca^{2+}-induced Ca^{2+} release (CICR) in intact and semi-intact systems since it is membrane permeant (Ehrlich & Bezprozvanny, 1994). Caffeine significantly increased the responsiveness of Merkel cell receptors to mechanical stimulation at the concentrations that stimulate intracellular Ca^{2+} release. A transient increase in spontaneous firing was observed in both St I and St II, in spite of a concomitant inhibition of stimulated responses in the St II. This is probably a manifestation of the tendency of caffeine to increase Ca^{2+} influx (Bianchi, 1962). It is,

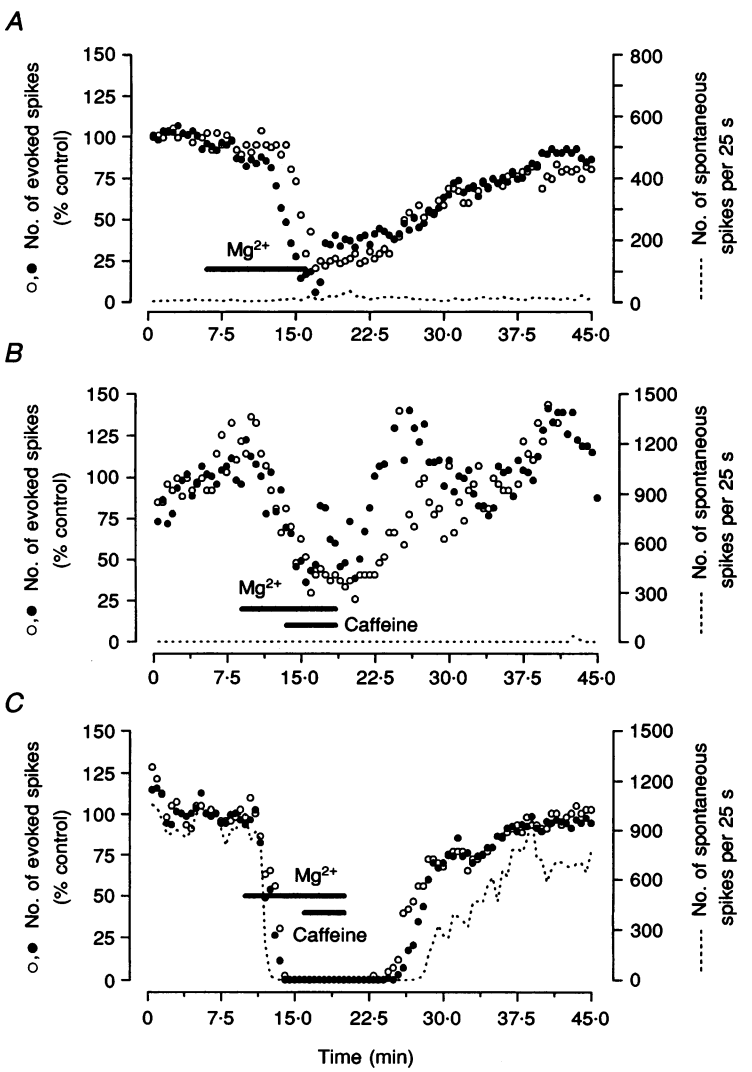

Figure 2. Effect of caffeine on St I requires Ca^{2+} influx

Mg^{2+} (2·5 or 5 mM) was used to inhibit competitively Ca^{2+} influx in St I receptors. ○, dynamic counts; ●, static counts. *A*, Mg^{2+} (2·5 mM for 10 min) blocked St I responses to mechanical stimulation in an St I receptor. Static counts were more sensitive to inhibition by Mg^{2+} than dynamic responses. *B*, after 5 min of 2·5 mM Mg^{2+} superfusion, caffeine (10 mM) was added for another 5 min. When caffeine was added before complete inhibition of responses, caffeine transiently and partially reversed the Mg^{2+}-induced inhibition. *C*, when caffeine was added after complete inhibition (by 5 mM Mg^{2+}) no effect was seen. When present, spontaneous spikes were also blocked by Mg^{2+}.

however, unlikely to be a significant factor in the demonstrated caffeine effects on St I function. Apart from the fact that the low frequency spontaneous firing was not always observed, it was short lived, usually decreasing or stopping before discontinuation of caffeine superfusion.

Caffeine increases cytosolic cAMP by inhibition of phosphodiesterase (Kumbaraci & Nastuk, 1982) but this typically occurs at lower concentrations of caffeine (0·1–1 mM; Smellie, Davis, Daly & Wells, 1979; Sawynok & Yaksh, 1993). The effective concentrations of caffeine used in this study (1–20 mM) are more in keeping with Ca^{2+} release (Sawynok & Yaksh, 1993; Ehrlich & Bezprozvanny, 1994). The membrane-permeable analogue of cAMP, 8-bromo-cAMP, had no appreciable effect on the responses of the receptors

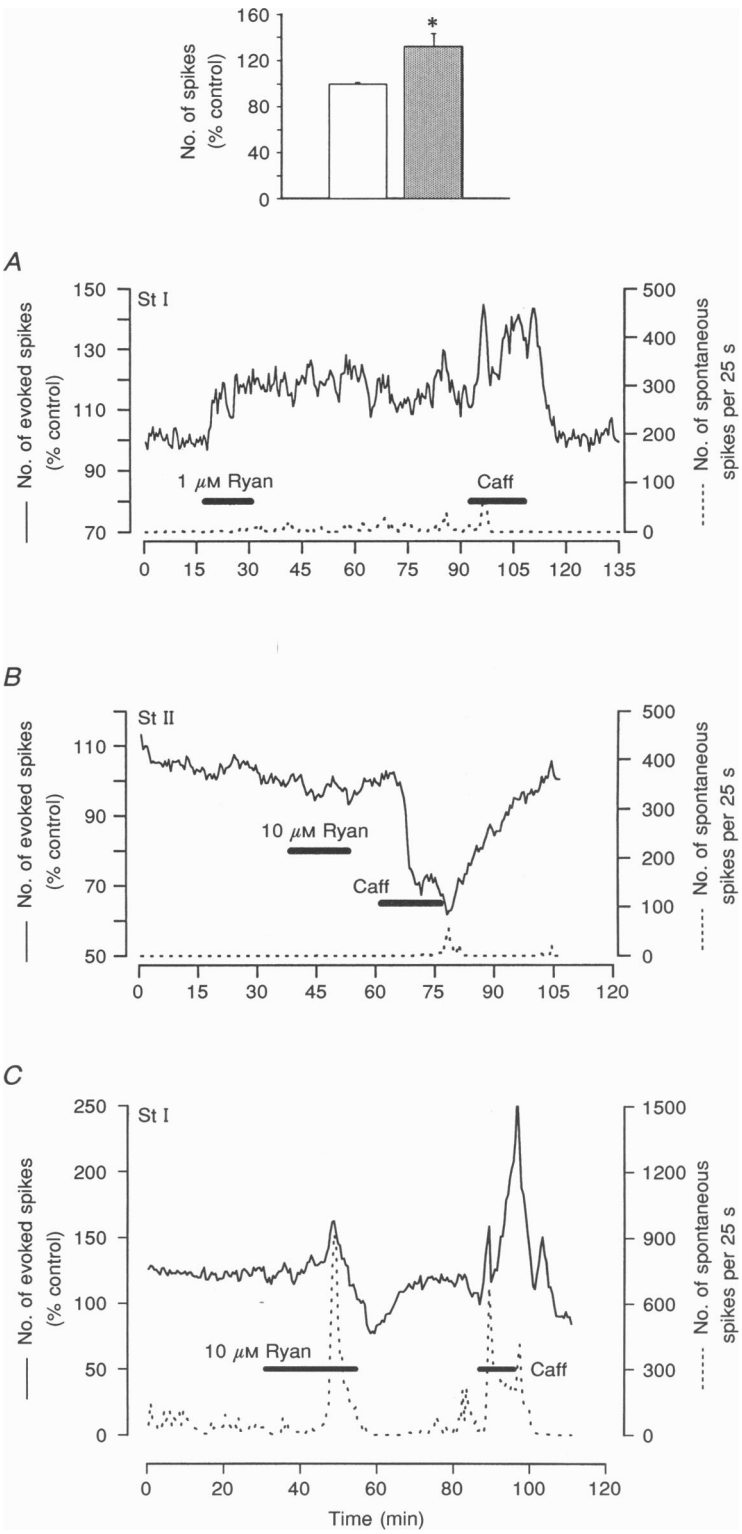

Figure 3. Effect of ryanodine

A, representative St I receptor showing the effect of superfusion of ryanodine (Ryan; 1 μM) for 15 min. Responses to mechanical stimulation were increased. Return to baseline was slow. Superfusion of caffeine about 60 min after ryanodine still increased responses but the increase was less than the average seen without prior ryanodine treatment (cf. Fig. 1). Inset, summary of five experiments showing that 1 μM ryanodine superfusion (▩) increased St I responses by 32·0 ± 10·9% of control (□). $^*P < 0·05$. *B*, superfusion of ryanodine (10 μM for 20 min) had no effect on the function of a typical St II. The receptor responded to caffeine (Caff) treatment as expected (cf. Fig. 1*C*) after ryanodine treatment. *C*, a higher concentration of ryanodine (10 μM) increased St I responses only briefly and could not be sustained, crashing below baseline values.

in our preparation (S. Senok & K. I. Baumann, unpublished observations). It is therefore unlikely that cAMP elevation is responsible for the observed actions of caffeine.

Caffeine action requires Ca^{2+} influx

Caffeine was able to partially reverse, or at least pause, the progression of Mg^{2+}-induced inhibition of St I receptor function if added before complete inhibition was achieved by Mg^{2+}. Since Mg^{2+} is a well-known competitive inhibitor of Ca^{2+} influx, and known to inhibit SA I receptor responses (Pacitti & Findlater, 1988; Baumann, Tsu & Yung, 1993), this suggests that some Ca^{2+} influx was required for caffeine to exert its effect on St I receptors. This is expected in a CICR system. It is noteworthy that Mg^{2+} also blocks the

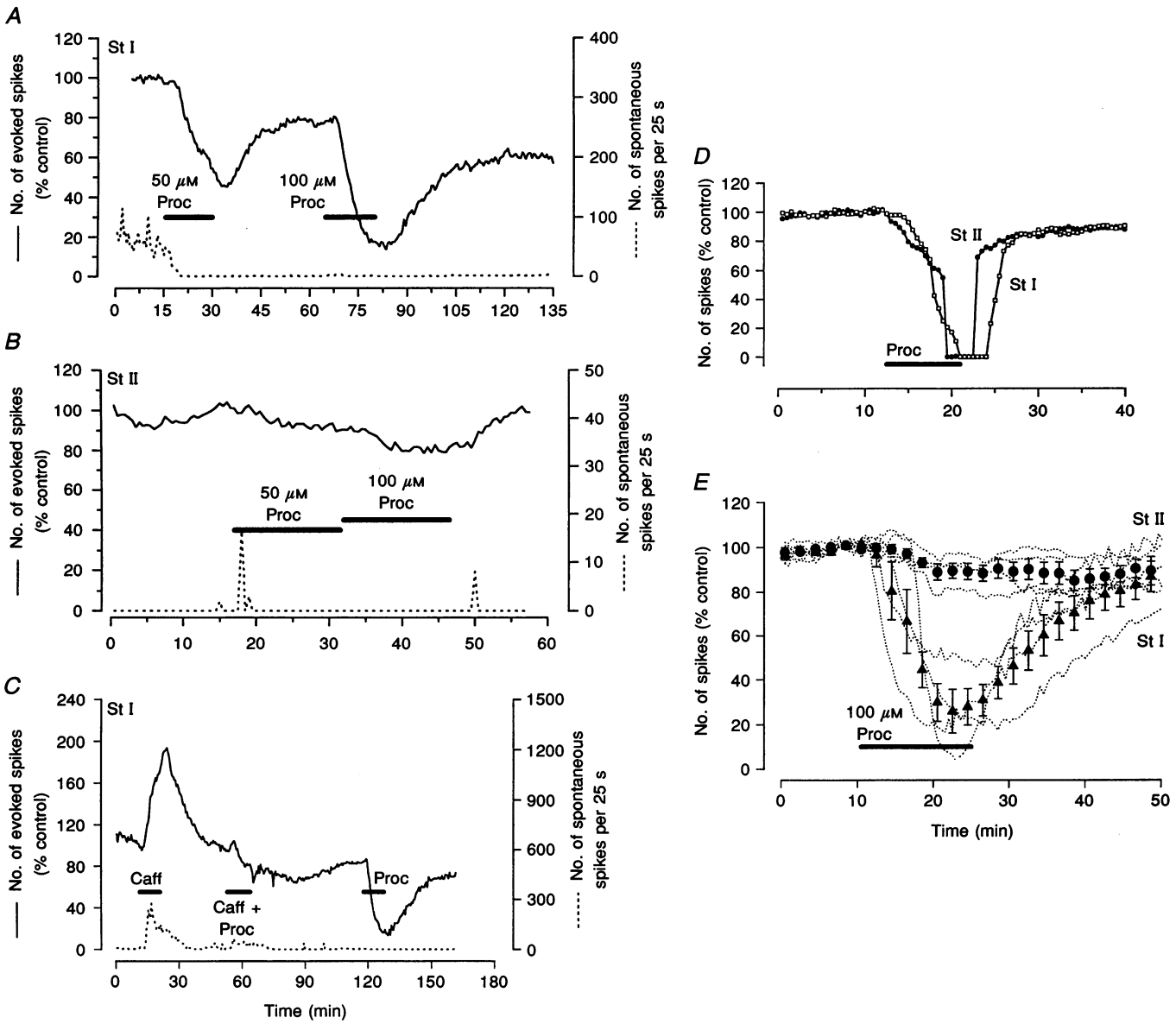

Figure 4. Effect of procaine

A, typical St I receptor response to procaine hydrochloride (Proc) superfusion. Inhibition of response to mechanical stimulation was concentration dependent with slow recovery. Spontaneous firing was also blocked. *B*, St II receptor superfused with 50 μM procaine for 15 min (no response) and switched to 100 μM for a further 15 min with only a salutary effect. Responses returned to baseline relatively quickly on removal of procaine. *C*, when procaine (100 μM) and caffeine (10 mM) were superfused concurrently on an St I, their net effects were not observed. *D*, higher concentrations of procaine (300 μM St I, 500 μM St II) blocked both St I and II function. The time courses were, however, quite different. Procaine is presumed to be blocking nerve conduction at the higher concentrations. *E*, procaine (100 μM) superfusion for 15 min inhibited St I responses by $75{\cdot}0 \pm 9{\cdot}1\%$ of control ($n = 4$) while St II receptors were inhibited by $10{\cdot}0 \pm 3{\cdot}3\%$ ($n = 4$). The individual traces of the experiments summarized show no overlap between responses of the two receptor types to procaine.

RyR in its own right (Meissner, 1994; Pozzan *et al.* 1994). In an intact preparation such as the one used in this study, Mg^{2+} would be expected first to block competitively Ca^{2+} influx before exerting any intracellular CICR blocking action. This might indeed explain the reported greater sensitivity of frog Merkel nerve endings (Ft I) to Mg^{2+} blockade than frog type II (Ft II) slowly adapting mechanoreceptors (Yamashita, Ogawa & Taniguchi, 1986).

The plant alkaloid ryanodine binds to the RyR channel and locks it in the open state, thus increasing the release of intracellular Ca^{2+} from the stores. At higher (10 μM) concentrations, ryanodine would deplete the stores and thus effectively antagonize CICR (Ehrlich & Bezprozvanny, 1994; Pozzan *et al.* 1994). We found that while ryanodine increased St I responses at 1 μM, the increase was not sustained at 10 μM with responses dropping below baseline. This is suggestive of depletion of the intracellular stores and is consistent with the presence of a CICR step in the transduction process of St I receptors.

Procaine hydrochloride, better known for its action as a local anaesthetic, is used as a specific inhibitor of CICR (e.g. Galione, Lee & Busa, 1991; Khoyi *et al.* 1993). Further evidence in support of a CICR process in St I was obtained using procaine. It was found to inhibit St I function at micromolar concentrations that had little or no effect on St II receptor responses. Since both Merkel cell and St II receptors have myelinated afferent fibres of similar calibre, it is unlikely that procaine was blocking impulse conduction in the nerve as a local anaesthetic would, but is exerting its specific CICR blocking action in the Merkel cell receptor while sparing the St II. The finding that procaine directly prevented the effect of caffeine when they were superfused together is also significant in this regard. The CICR blocking action of procaine in other tissues requires millimolar concentrations (e.g. Khoyi *et al.* 1993; Huang, 1995). In St I receptors, superfusion of 50–100 μM procaine was sufficient to block responses. Higher concentrations (300–500 μM) apparently blocked nerve conduction as well since St II function was also blocked. Recovery of St II responses after inhibition by procaine was much faster and rather abrupt. It is conceivable that as a weak base (pK_a 8·9) procaine may be taken up and concentrated by Merkel cells (de Duve, de Barsy, Poole, Trouet, Tulkens & Hoof, 1974), thus achieving the concentrations required to block CICR. The slow recovery of St I responses after procaine treatment would seem to support this.

$Ins P_3$ receptor-mediated transduction is unlikely in St I

Caffeine has been shown to inhibit intracellular Ca^{2+} mobilization through the inositol 1,4,5-trisphosphate ($Ins P_3$) second messenger pathway in *Xenopus* oocytes (Parker & Ivorra, 1991), cerebellar microsomes (Brown, Sayers, Kirk, Michell & Michelangeli, 1992), and permeabilized smooth muscle cells (Hirose, Iino & Endo, 1993). Caffeine at 10 mM was sufficient to cause almost complete inhibition of $Ins P_3$ action (Bezprozvanny, Bezprozvannaya & Ehrlich, 1994). It is thus unlikely that Ca^{2+} release from the $Ins P_3$-sensitive pool plays any significant role in the transduction of Merkel cell receptors.

Mechano-electrical transduction in Merkel cell receptors

In a model of mechano-electrical transduction in the cutaneous Merkel cell receptor (SA I), Iggo & Findlater (1984) proposed that mechanical distortion of the filamentous processes of the Merkel cell led to alteration of membrane permeability of the epidermal surface of the cell, resulting in Ca^{2+} entry and subsequent mobilization of the dense cored granules and release of the putative transmitter substance. Following the description of three types of ionic channels in isolated Merkel cells, Yamashita *et al.* (1992) suggested that mechanical deformation of Merkel cells activated putative mechano-sensitive (MS) channels which then generated a depolarizing potential leading to activation of the L-type Ca^{2+} channel which they found in Merkel cells. Chan, Yung & Baumann (1996) recently reported cytosolic Ca^{2+} elevations in Merkel cells in response to mechanical stimulation as well as high-K^+ depolarization. This suggests the presence of MS channels and supports the report of voltage-gated Ca^{2+} channels in Merkel cells by Yamashita *et al.* (1992). There is, however, as yet no experimental proof for the presence of MS channels in Merkel cells. The transduction mechanism in this receptor has thus remained unclear, wherever the site of transduction may be.

The question of Ca^{2+} and Merkel cells has been a rather vexing one. Though it has been shown that Ca^{2+} influx is required in the transduction process of Merkel cell receptors (e.g. Yamashita *et al.* 1986; Pacitti & Findlater, 1988; Baumann *et al.* 1993), the specific organic blockers of voltage-gated Ca^{2+} channels like verapamil and ω-conotoxin GIVA have had unconvincing actions on the function of the receptors. Rather high concentrations are required for appreciable inhibition. In the isolated skin–nerve preparation, Baumann *et al.* (1993) found that the 100 μM concentration of verapamil required to block responses also blocked nerve conduction in the preparation. Furthermore, the L-type Ca^{2+} channel opener Bay K 8644 had only a salutary effect on receptor function. Only the inorganic Ca^{2+} channel blockers like Mn^{2+} and Mg^{2+} consistently block receptor function.

Two phases of Ca^{2+} influx

We speculate that Ca^{2+} influx into Merkel cell receptors occurs by two mechanisms: through the putative MS channels activated by mechanical deformation and through the voltage-dependent Ca^{2+} channel (Yamashita *et al.* 1992). Ca^{2+} influx through the MS channel probably contributes mainly to the dynamic responses to mechanical stimulation and hence might explain the high dynamic sensitivity of the receptor. MS channels are known to inactivate fairly rapidly and are unusually voltage sensitive (Hamill & McBride, 1992; Sukharev, Martinac, Arshavsky & Kung, 1993). The

depolarization following ionic influx through the MS channel would therefore be expected to also inactivate it. Thus sustained mechanical displacement will not keep the channels open long enough for the typical slowly adapting response of Merkel cell receptors. The second phase of Ca^{2+} influx probably occurs through activation of the high threshold Ca^{2+} channel as suggested by Yamashita *et al.* (1992), and may contribute more to the sustained responses during the static phase of mechanical stimulation. This study found that caffeine (and procaine) exerted the most effect on the static phase. CICR may therefore be more important for the slowly adapting response of the receptor.

There are no previous reports of the involvement of CICR in mechano-sensory transduction. CICR would seem superfluous in a nerve terminal which simply needed to generate a receptor potential in response to mechanical stimulation. In cochlear hair cells, where cytosolic Ca^{2+} elevation is part of the response to mechanical stimulation, this is associated with receptor adaptation (Torre, Ashmore, Lamb & Menini, 1995). If CICR were to play a role in such a system, it would diminish rather than increase the responsiveness of the mechanoreceptor to sustained stimulation as seen in Merkel cell receptors. It is therefore conceivable that this might indeed be evidence that a Ca^{2+}-dependent process like synaptic transmission is present in Merkel cell receptor transduction.

In conclusion, this study has found functional evidence that a CICR step is involved in the mechano-electrical transduction process of Merkel cell receptors. Taken together with the reported effects of chloroquine on the function of St I receptors and morphology of Merkel cells, the CICR process may reside in Merkel cells rather than the nerve endings. Thus our results are in support of the hypothesis that the Merkel cells are mechano-electrical transducers in this vibrissal slowly adapting mechanoreceptor.

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Acknowledgements

This work was supported by the Research Grants Council, Hong Kong.

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Received 21 October 1996; accepted 3 January 1997.