

Evidence for glutamate receptor mediated transmission at mechanoreceptors in the skin

B. Matthew Fagan and Peter M. B. Cahusac^{CA}

Department of Psychology, University of Stirling, Stirling FK9 4LA, UK

^{CA}Corresponding Author

Received 27 October 2000; accepted 22 November 2000

The functional role of Merkel cells in the mechanosensitivity of the slowly adapting type I responses has been a controversial issue for many years. Here we show, for the first time, that glutamate receptor-mediated transmission is largely responsible for the static component of the slowly adapting type I response. An isolated sinus hair preparation was used to study the two types (I and II) of slowly adapting units. A broad spectrum ionotropic glutamate receptor antagonist kynurenatate (1–10 mM) caused reliable and dose-dependent reductions in

the static component of type I unit responses to mechanical stimulation. In addition, an amino acid transmitter candidate aspartate applied to the preparation selectively increased responses in type I units but not responses in type II units. This evidence establishes that the Merkel cell is a mechano-electric transducer, and challenges prevailing views that the Merkel cell acts merely as a support or target cell in the epidermis. *NeuroReport* 12:1–7 © 2001 Lippincott Williams & Wilkins.

Key words: Excitatory amino acids; Kynurenatate; Merkel cell; SAI; Slowly adapting mechanoreceptor; Synapse; Type I mechanoreceptor

INTRODUCTION

In 1875 Friedrich Sigmund Merkel [1] first described cells in the epidermal layer of the skin that were later named after him. His observation that these cells were closely apposed to nerve terminals led him to describe them as 'Tastzellen' (touch cells). However the precise role of Merkel cells remains controversial [2–5]. Merkel cells have been implicated as targets for developing peripheral nerves and as elements of control in the regulation and maintenance of the epidermis [2,6], but there is also compelling evidence that Merkel cells act as mechanoelectric transducers, i.e. they are mechanoreceptors [3].

Slowly adapting (SA) mechanoreceptor units are distinguished by their sustained response to static mechanical stimuli applied to the skin or hair follicle. They are further categorized into type I and type II units depending upon their sensitivity and pattern of firing. These receptors in the skin are referred to as SA I and SA II respectively, and in sinus hair capsules as St I and St II. The importance of slowly adapting type I units is emphasized by studies in primates, including humans, which show they are responsible for fine spatial pattern discrimination by the fingertips [7].

Iggo and Muir [8] first correlated the presence of the Merkel cell–neurite complexes with the neural responses of SA I mechanoreceptors. Dense cored vesicles are concentrated in the Merkel cell's cytoplasm adjacent to a synaptiform junction made with the nerve terminal. The vesicles were observed to fuse with the membrane and under

hypoxia conditions their reduced numbers was correlated with SA I receptor failure [9], a lysosomotropic agent which may inhibit the processing and/or release of a transmitter from these vesicles and shown to interfere with calcium dependent processes, disrupts the structure and distribution of the vesicles, correlating with a selective dose-dependent impairment of St I responses [10].

Further evidence for an active synapse within the Merkel cell–neurite complex has been obtained from studies showing that type I responses were selectively reduced by Ca²⁺ blockers. Voltage-gated Ca²⁺ channels were also reported in Merkel cells (for review see [5]). Recently, cytosolic calcium was shown to increase in Merkel cells during their mechanical stimulation or exposure to hypotonic solutions [11]. Despite this evidence, all attempts to block chemical transmission have failed [8,12,13]. However, none of those studies attempted to block excitatory amino acid transmission. There is now compelling evidence that an excitatory amino acid acts as a neurotransmitter at the first peripheral synapse in the visual [14] and auditory [15] systems. We therefore endeavoured to test whether a similar transmitter acts at the Merkel cell–neurite complex junction.

We used an isolated rat vibrissal preparation appropriate for the study of single isolated mechanoreceptor unit responses [16]. The preparation allows easy access of pharmacological agents to the mechanoreceptors responsible for St I and St II unit responses. St II units acted as controls since it is known that their activity does not

depend on synaptic transmission. We studied the effects of kynurenat, a broad spectrum ionotropic glutamate receptor antagonist, on responses from both types of units.

MATERIALS AND METHODS

The isolated rat sinus hair preparation developed by Baumann and colleagues [16] was used. Permission to use animals in these experiments was provided by the Stirling University Department of Psychology's Ethics Committee. Sinus hairs from 36 Wistar-derived rats had the distal end of the deep vibrissal nerve removed by micro-dissection from an excised whisker pad. While immersed in synthetic interstitial fluid (SIF) the capsule was slit longitudinally. The sinus hair was then mounted on a Silguard platform in a custom made organ bath (obtained from Professor Baumann). Insect pins kept the hair in position and held the slit sides of the capsule apart ensuring ready access of SIF (and drugs) to receptors within the sinus hair. The nerve was stripped of its outer sheath and fine strands of nerve attached to the silver recording wire situated in a layer of Fluorinert (Sigma) at the bottom of the bath.

Nerve strands were tested for mechanosensitivity by manual displacement of the hair shaft with fine forceps, and electrical activity monitored on an oscilloscope and loud speaker. Once single units were obtained, a feedback-controlled mechanical transducer [17] was used, with its probe attached to the end of the hair shaft 5–10 mm from the hair bulb. The transducer was calibrated to produce a range of displacements between 0.5 and 1.5 mm. Stimulus parameters were used that produced ~75% of maximal response. Stimuli were applied every 15–30 s. Each stimulus consisted of 500 ms onset and offset ramps, and a 4 s plateau (total duration 5 s). Discriminated action potentials were recorded on a Cambridge Electronic Design 1401+ laboratory interface and computer. The two different types of slowly adapting units were readily distinguished by their firing patterns. St I units had a characteristic irregular firing pattern, in which inter-spike intervals (ISIs) of static responses showed a wide and positively skewed distribution (adequately described as a U distribution). The coefficient of variation (COV) was thus always >0.1. In contrast St II units had highly regular ISIs and a corresponding COV <0.1. The ratio between dynamic and static responses was also different for the two types of unit, being significantly higher in St I units. On occasion multiple units were recorded onto tape and analysed off-line using spike template-matching software (CED Spike2).

Drugs were applied to the preparation mixed in a SIF solution and balanced to pH 7.4. Kynurenat was applied at concentrations up to 10 mM, a level known to displace kainate binding and block synaptic transmission *in vitro* (e.g. in the hippocampal slice preparation [18]). The 20 ml solution was introduced into the bath at 1 ml/min as the unit was stimulated and responses recorded. Sections of the data were also recorded in analog format onto video tape for off-line analysis. A test using caffeine at 10 mM was used to distinguish between the two types of units. St I units were known to show increased responses to caffeine, while St II units show decreased responses [17,19]. This test was used, where possible, to confirm the type of unit at the end of an experiment. The rapid depressant action of caffeine on St II unit responses indicated that

drugs can also gain easy access to these receptors in this preparation. This makes the interpretation of differential drug actions on the two types of units unambiguous, and effects cannot easily be explained away by differential diffusional barriers.

The spike counts were converted into spike/s. Some of these data (at specified drug dose) were analysed using one-way repeated measures analysis followed by simple contrasts between the three testing times: Before, During kynurenat and Recovery (see Table 1). For linear regression analyses the response data for dynamic and static components were corrected for the spontaneous rate by subtracting the mean spontaneous firing rate recorded 3 s prior to each ramp onset. The responses for dynamic and static components During kynurenat were then transformed into percentage effect relative to control using: during response rate \times 100% control response rate.

RESULTS

Well isolated extracellular spike recordings were made from both St I and St II units. The irregular response to a mechanical stimulus by the St I unit (Fig. 1a) contrasts with the highly regular response of the St II unit (Fig. 1b). On occasions when two or more units were recorded simultaneously, post-experiment analyses of spikes were performed by off-line template matching (Fig. 2). This allowed direct comparison of the effects of kynurenat on St I and St II units recorded together.

The effects of kynurenat applied in the range 1–10 mM were studied in 24 St I units. An example from one experiment is shown in Fig. 3. Kynurenat reduced both

Table 1. Effects of kynurenat at the highest doses (5 mM and 10 mM) on St I ($n=12$) and St II units ($n=6$) (equal numbers of experiments per dose). The figures given are the mean firing rates in spikes/s (\pm s.e.m.).

	Before	During kynurenat	Recovery
St I			
Dynamic	56.9 \pm 10.1	*39.1 \pm 8.9	54.7 \pm 11.0
Static	25.9 \pm 7.1	**9.9* \pm 6.2	17.0 \pm 6.9
Spontaneous	2.3 \pm 0.9	0.9* \pm 0.4	1.9 \pm 0.7
St II			
Dynamic	58.5 \pm 3.6	55.6 \pm 2.8	53.8 \pm 3.9
Static	33.2 \pm 5.5	33.6* \pm 5.5	32.8 \pm 5.5
Spontaneous	6.0 \pm 6.0	6.3 \pm 6.3	6.8 \pm 6.3

A strong and highly statistically significant effect of kynurenat (During kynurenat) was observed on the St I static component. The dynamic response showed a smaller reduction, while spontaneous also showed a small reduction. Recovery in the response for each component was observed. The firing of St II units were unaffected by kynurenat, although there was a small reduction in the dynamic component during recovery compared with before.

Repeated measures ANOVA on each of the components produced highly significant effects for St I units across the three testing times for the static response ($F(2,22)=9.45$, $p=0.001$). Less significant results were achieved for the dynamic response ($F(1,2,12.9)=4.8$, $p=0.043$), and failed to reach significance for the spontaneous activity ($F(2,22)=3.27$, $p=0.057$). None of the St I before with recovery contrasts were more significantly different than at the 0.025 level. Similar ANOVA on St II unit data revealed that only the before versus recovery conditions were significant ($p=0.012$), suggesting a general decline in responsiveness over time and not a drug effect. Explanation of statistical significance following the ANOVA using contrasts between testing times: *rate indicates significantly different from before at $p<0.025$; rate* indicates significantly different from recovery at $p<0.025$; **rate indicates significantly different from before at $p<0.01$.

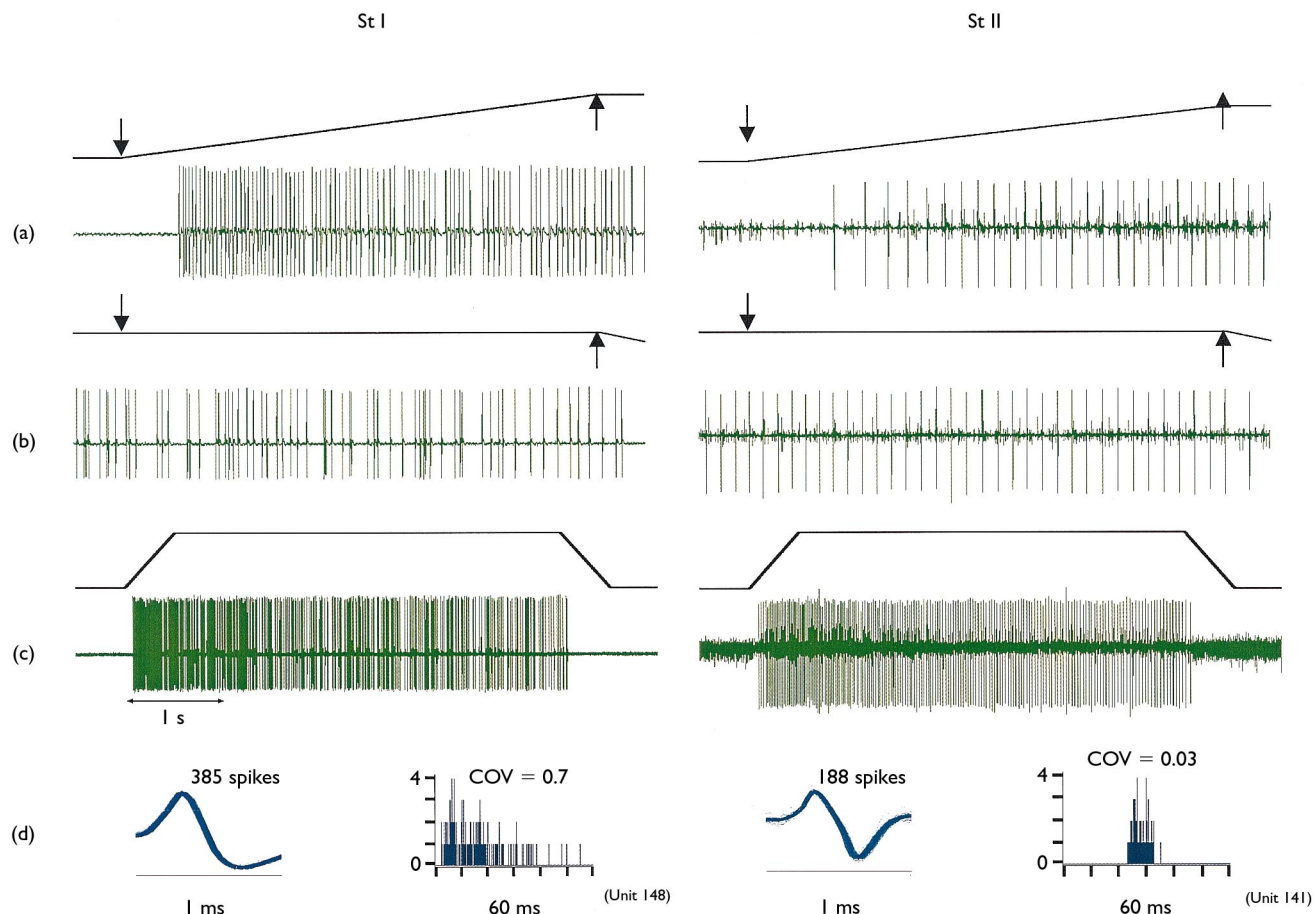


Fig. 1. Sample oscilloscope spike records from St I (left) and St II (right) units during periods used for analysis of responses. Above each trace is shown the stimulus ramp consisting of a 1 mm deflection. The dynamic response period for each type of unit is shown in (a) (during the 0.5 s from onset to plateau as indicated between the two arrows on the ramp trace). The static response period for each type of unit is shown in (b) (during the last 1 s of the plateau as shown between the two arrows on the ramp trace). Note the difference in regularity of firing between the St I and St II records. (c) Whole responses from onset to offset of the ramp stimulus for both types of unit. The actual spike shapes (delay line overdrawing of repeatedly sampled spikes) and the inter-spike interval histograms are shown in (d). For the St I unit spike shapes, 385 spikes were sampled, and for the St II unit 188 spikes were sampled. The St I unit had a high COV of 0.7 (indicative of high inter-spike interval variability) and the St II unit had a COV of 0.03. The noise level (below identifiable unit activity) on all the spike records was $\sim 15 \mu\text{V}$.

the static and dynamic response components. A plot of the percentage effect for the static and dynamic components against log dose of kynurenatate revealed diverging lines (Fig. 4), indicating that the static component was most sensitive to the antagonist. Regression analyses revealed a significant linear relationship between the static response and log dose (slope $b = -42.8$, $p = 0.024$, $r^2 = 21\%$). In contrast, the linear relationship between the dynamic response and log dose failed to reach statistical significance ($b = -19.0$, $p = 0.25$, $r^2 = 6\%$). A summary of spike firing rates from experiments involving the two highest doses of kynurenatate, 5 mM and 10 mM, is given in Table 1.

There was virtually no effect of the two highest kynurenatate doses tested, 5 mM and 10 mM, on St II unit responses ($n = 6$; Fig. 5; Table 1). In two cases simultaneous recordings were made from a St I and a St II unit. Post-experiment (off-line) analysis using template matching (Fig. 2) demonstrated that only responses from the St I units were affected by the antagonist.

Some of the reductions in St I unit responses might have been due to slow deterioration of the preparation during the course of drug application and recovery. In four experiments St I units were studied for the same amount of time but no drug was delivered to the preparation. During the time when drug effects would be expected (had the drug been delivered), there was a small reduction in both the dynamic (median of control = 90%, Wilcoxon test, $p = 0.068$) and static (median of control = 91%, $p = 0.46$) components. These reductions were clearly less than the dose dependent effects of kynurenatate (Fig. 4), but may explain the often poorer than expected recovery back to control levels of responsiveness following drug applications.

In order to provide additional evidence that an amino acid transmitter operates at the Merkel cell-neurite junction, we applied a candidate excitatory amino acid, aspartate. This amino acid is known to act on NMDA and other glutamate receptors [20]. The application of aspartate at

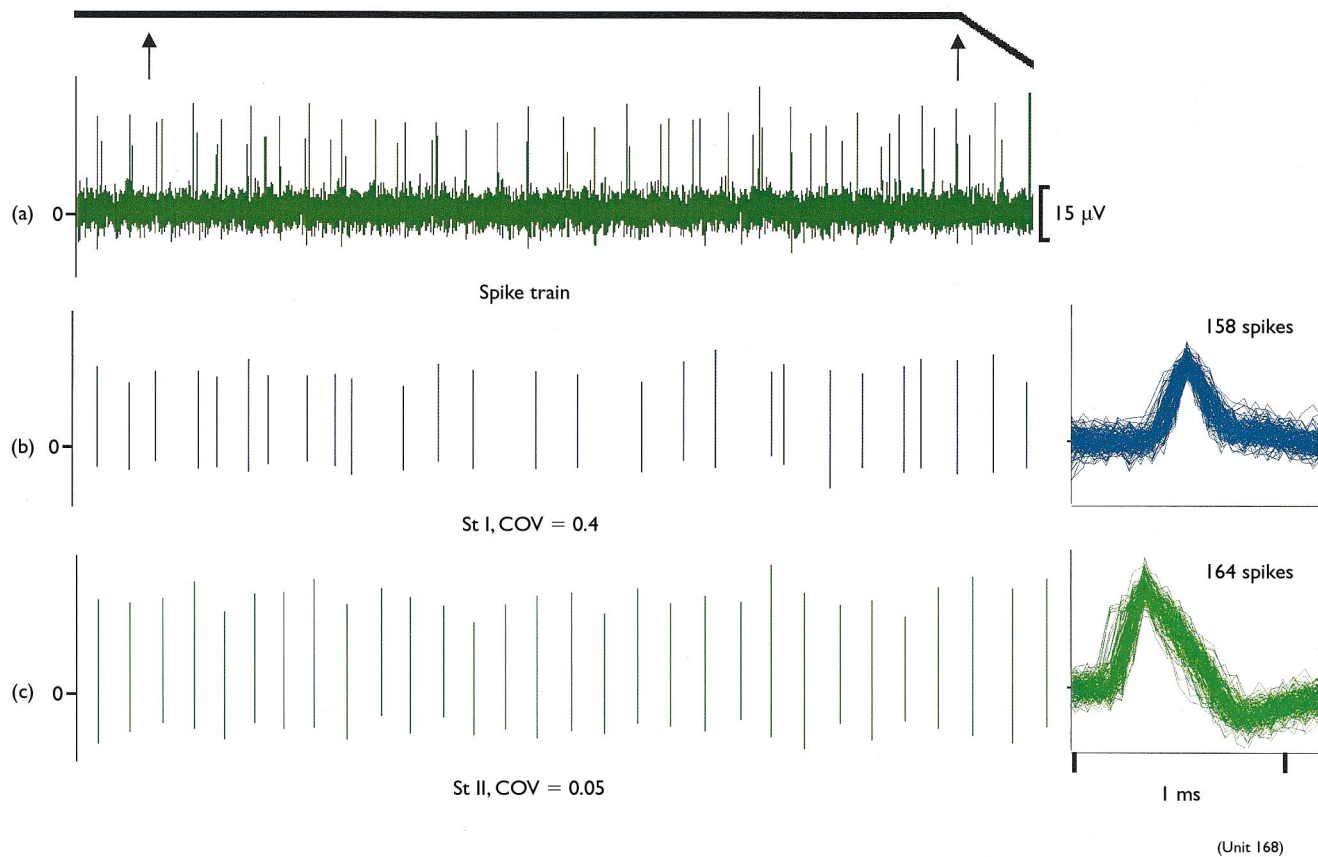


Fig. 2. A sample oscilloscope spike record from one experiment where two different spike units (one St I, the other St II) were identified using post-experiment off-line template matching. The top record (a) shows the original spike record with the ramp trace above taken near the end of the plateau (1 s window for analysis indicated by upright arrows). (b) shows the St I unit (COV = 0.4) and delay line spike shape (overdrawing of repeatedly sampled spikes) shown to the right. In (c) the same information is shown for the discriminated St II unit (COV = 0.05). Deflection of the ramp from baseline was 0.5 mm. Off-line analyses are performed at the end of the experiment and contrast with on-line analyses which are done in real time during the experiment.

10 mM produced increases in spontaneous and evoked responses in four St I units (median of control = 133%), whereas in three St II units there was no effect (median of control 100%). There was a statistically significant difference between the two groups (Mann-Whitney $U = 0$, $p = 0.034$).

DISCUSSION

This study has demonstrated that a broad spectrum ionotropic glutamate receptor antagonist reliably reduced evoked responses of St I units. A greater effect was seen on the static response component. The reduction was dose dependent and reversible. In contrast, St II units were virtually unaffected by the antagonist. It is unlikely that the selective effects of kynurenate on type I responses is due to a blockade of non-synaptic glutamate receptors for two reasons. First, excitatory amino acids are not known to exert tonic effects by low-level extracellular circulation since there are high-affinity rapid transporter mechanisms [21]. Second, it is difficult to explain how the blockade of low level non-synaptic glutamate should differentially affect the static response component compared with the dynamic component. The selective excitatory effects of aspartate on type I responses is consistent with the functional location of excitatory amino acid receptors on

type I unit nerve endings. It remains to be determined which kind of ionotropic glutamate receptors are preferentially implicated.

These data strongly support the hypothesis that the Merkel cell acts as a transducer, releasing an amino acid to traverse the junction to the primary afferent nerve terminals. The data are also consistent with other recent work suggesting a dual process model [5,22] in the generation of type I unit responses: mediation of the high frequency dynamic response component by the nerve terminals, and mediation of the static response component by Merkel cells. A preferential effect on the static versus dynamic component of type I responses was found during Ca^{2+} blockade [5]. Later work showed that caffeine had a greater effect in enhancing static compared with dynamic responses [17]. Further, the different effects of cathodal and anodal electrical stimulation of type I units were similar to those found for inner hair cells [22] suggesting that, like the inner hair cell, at least part of the type I response involves synaptic transmission. In the salamander and *Xenopus* the responses from Merkel cell-neurite complexes are rapidly adapting, consistent with mediation by nerve terminals, and thus were not affected by treatments altering synaptic transmission such as replacement of Ca^{2+}

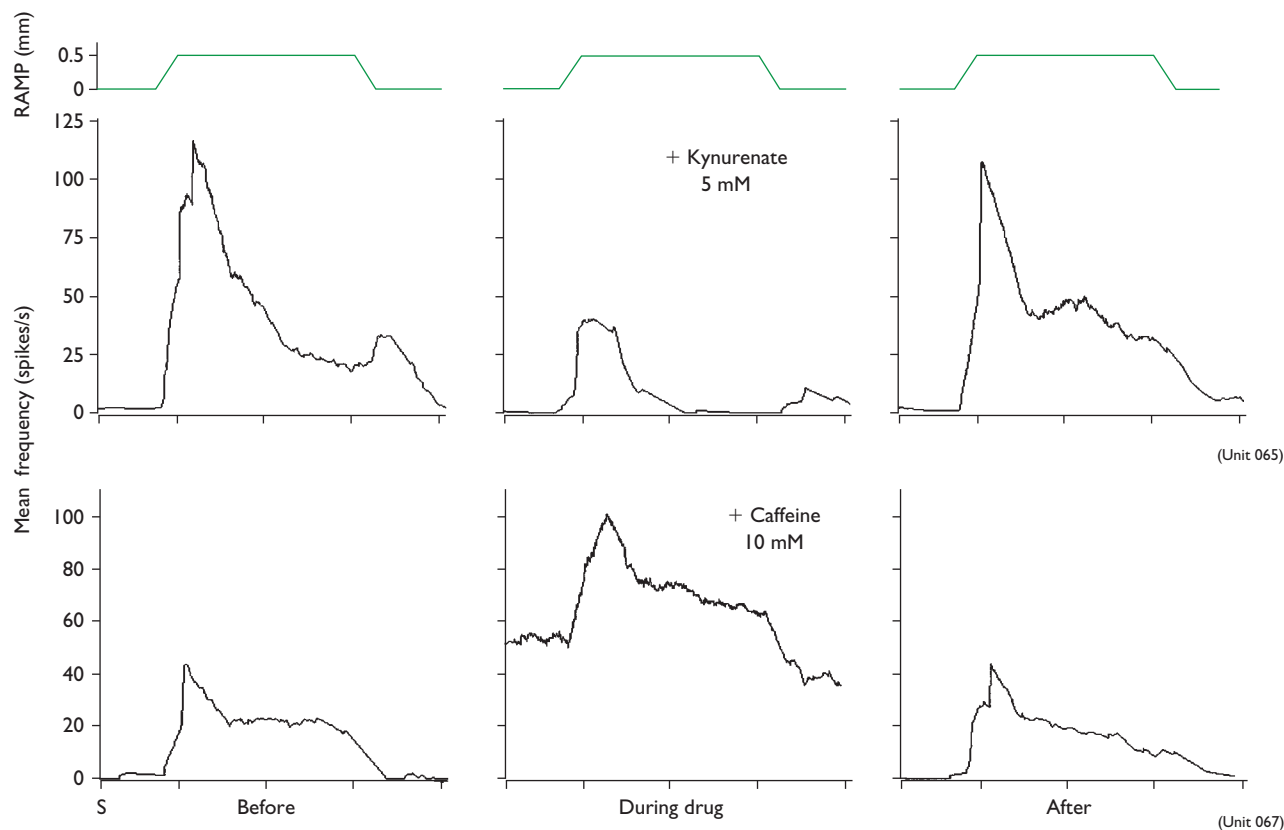


Fig. 3. The effects of kynurenatate and caffeine on St I unit responses. Top three records show Before drug administration (left), During drug 5 mM kynurenatate (middle) and recovery after flushing with SIF. Note the reduction by kynurenatate of the dynamic and static components. The lower three records show the results obtained from a different unit using 10 mM caffeine (applied at the during drug stage). The caffeine resulted in an elevation of spontaneous firing and evoked responses.

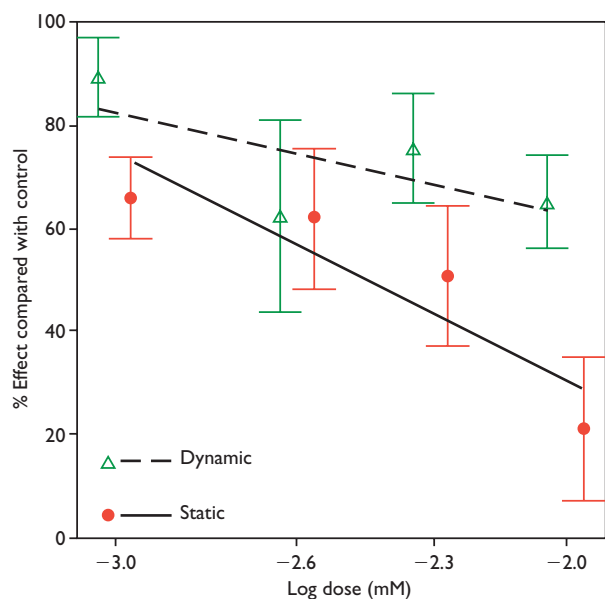


Fig. 4. Log dose-response relationship for kynurenatate (range 1–10 mM) on dynamic (triangles) and static (circles) components of the response. The mean percentage of control response is plotted \pm s.e.m. The static component appears to be more reduced by kynurenatate than the dynamic component, as confirmed by statistical analyses detailed in the text. Each point represents data from six experiments.

with Mg^{2+} or by temperature changes [2]. The phase-locked responses of cat St I and SA I units [23] observed to vibratory stimulation of >1 kHz, representing dynamic activity, would therefore also be generated by the nerve terminals. Finally, developmental evidence showed that sustained responses to tactile stimulation in newborn kittens could only be obtained when Merkel cells appeared in the epidermis [24]. In our study, using the adult rat sinus hair, $>20\%$ of the response remains during kynurenatate blockade: this may represent the contribution due to activity generated by the nerve. Alternatively, this residual response may be due to the existence of a co-transmitter or neuromodulator released from Merkel cells, or to the involvement of metabotropic glutamate receptors not blocked by kynurenatate.

It is likely that the type of Merkel cells associated with type I units comprise a subpopulation of a diverse class of cells whose developmental origin is uncertain [25,26]. Consistent with the conclusions from the present work are studies showing the existence of ionotropic glutamate receptors on peripheral axons in the skin [27] and the discovery of a glutamate-mediated signalling pathway in keratinocytes [28].

CONCLUSION

The functional role of Merkel cells as mechanoreceptors in the skin has been controversial. In this study carried out in

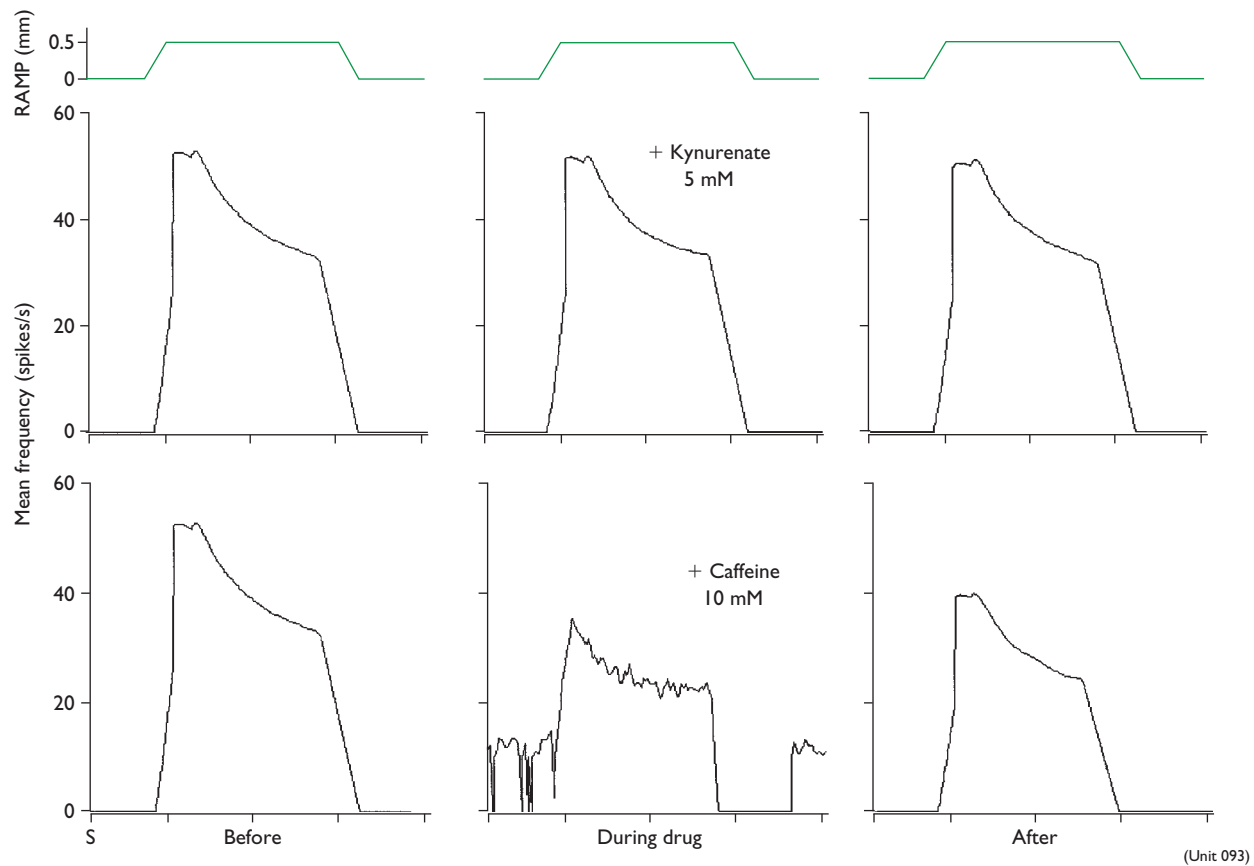


Fig. 5. Lack of effect of 5 mM kynureate on St II unit responses. Top three records show Before drug administration (left), during drug 5 mM kynureate (middle) and recovery After flushing with SIF. Both dynamic and static components were unaffected by kynureate. The lower three records show the results obtained from the same unit using 0.10 mM caffeine (applied at the during drug stage). The caffeine resulted in an elevation of spontaneous firing and a reduction in the evoked response.

the isolated vibrissa preparation, blockade of glutamate receptors resulted in a pronounced reduction of type I slowly adapting mechanoreceptor unit responses. This indicates that Merkel cells are mechanoreceptors and that a glutamatergic synapse exists between Merkel cells and their associated primary afferent nerve endings in the skin. The static responses of type I units were particularly susceptible to the blocking action of the antagonist. Taken together with evidence from other studies, the data suggest that Merkel cells code maintained tactile stimulation, while the afferent nerve endings code transient stimulation.

REFERENCES

- Merkel F. *Arch Mikrosk Anat* **11**, 636–652 (1875).
- Diamond J, Mills LR and Mearow KM. *Prog Brain Res* **74**, 51–56 (1988).
- Iggo A and Findlater GS. In: Hamann W and Iggo A, eds. *A review of Merkel cell mechanisms. Sensory Receptor Mechanisms*. Singapore: World Scientific Publishing Co; 1984, pp. 117–131.
- Tachibana T. *Arch Histol Cytol* **58**, 379–396 (1995).
- Ogawa H. *Prog Neurobiol* **49**, 317–334 (1996).
- Scott SA, Cooper E and Diamond J. *Proc Royal Soc B* **211**, 455–470 (1981).
- Johnson KO, Hsiao SS and Twombly IA. In: Gazzaniga MS, ed. *Neural Mechanisms of Tactile Form Recognition. The Cognitive Neurosciences*. Cambridge, MA: MIT Press; 1995, pp. 253–267.
- Iggo A and Muir AR. *J Physiol (Lond)* **200**, 763–796 (1969).
- Findlater GS, Cooksey EJ, Anand A *et al.* *Somatosens Res* **5**, 1–17 (1987).
- Senok SS, Halata Z and Baumann KI. *Neurosci Lett* **214**, 167–170 (1996).
- Baumann KI, Senok SS, Chan E and Yung WH. Calcium influx and calcium-induced calcium release in mechanically stimulated Merkel cells of rat sinus hair type I mechanoreceptors. In: Suzuki H and Ono T, eds. *Merkel Cells, Merkel Cell Carcinoma and Neurobiology of the Skin*. Amsterdam: Elsevier; 2000, pp. 73–81.
- Smith KR and Creech B. *J Exp Neurol* **19**, 477–482 (1967).
- Baumann KI and Chan E. *J Physiol (Lond)* **459**, 207P (1993).
- Thoreson WB and Ulphani JS. *Brain Res* **676**, 93–102 (1995).
- Ottersen OP, Takumi Y, Matsubara A *et al.* *Prog Neurobiol* **54**, 127–148 (1998).
- Baumann KI, Chan E, Halata Z *et al.* *Neurosci Lett* **213**, 1–4 (1996).
- Senok SS and Baumann KI. *J Physiol (Lond)* **500**, 29–37 (1997).
- Bortolotto ZA, Clarke VRJ, Delany CM *et al.* *Nature* **402**, 297–301 (1999).
- Fagan BM and Cahusac PMB. *J Physiol (Lond)* **520P**, 52P (1999).
- Stone TW and Burton NR. *Prog Neurobiol* **30**, 333–368 (1988).
- Kanner BI. *FEBS Lett* **325**, 95–99 (1993).
- Yamashita Y and Ogawa H. *Somatosens Mot Res* **8**, 87–95 (1991).
- Gottschaldt KM and Vahle-Hinz C. *Science* **214**, 183–186 (1981).
- Kasprzak H, Tapper DN and Craig PH. *Exp Neurol* **26**, 439–446 (1970).
- Hartschuh W and Weihe E. *Prog Brain Res* **74**, 181–187 (1988).
- Christie KN, Thomson C, Hopwood D *et al.* *Arch Oral Biol* **41**, 901–904 (1996).

27. Kinkelin I, Brockner EB, Koltzenburg M and Carlton SM. *Neurosci Lett* 283, 149–152 (2000).
28. Genever PG, Maxfield SJ, Kennovin GDF *et al.* *J Invest Dermatol* 112, 337–342 (1999).

Acknowledgements: To Dr R.H Evans, formerly of the Pharmacology Department, University of Bristol, for his bold suggestion many years ago to do these experiments. The University of Stirling for finding B.M.F.'s studentship. To Professor Klaus Baumann, of the University of Hamburg, for guidance and support in our work with the isolated sinus hair preparation and for his gift of an isolated tissue bath. The Department of Physiology, Chinese University of Hong Kong, for use of a mechanical transducer. The University of Stirling Animal Facility's excellent service.

AQ: Refs 3 and 7. Titles of chapters please.