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ACUTE EFFECTS OF NEOMYCIN ON SLOWLY ADAPTING TYPE I AND TYPE II CUTANEOUS MECHANORECEPTORS IN THE ANAESTHETIZED CAT AND RAT

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SUMMARY

1. Slowly adapting type I (SAI) and type II (SAII) mechanoreceptors in the skin were studied in anaesthetized cats and rats employing mechanical stimuli every 30 s. Individual stimuli rose within 200 ms to a plateau force which was kept constant through a feedback control unit for 2000 ms.

2. In cats, close arterial infusion of neomycin (2·5 mg/min) as sulphate was given through a side branch into the femoral blood stream for 5, 10 or 20 min at a rate of 0·025 ml/min. At other times saline was infused at the same rate.

3. After 20 min of neomycin infusion (total 50 mg) nervous discharge of cat SAI receptors was suppressed to about 30% of the control responses before neomycin infusion. Nervous responses were reduced more profoundly during the plateau phase of stimulation than during the dynamic phase. The interspike interval histogram was severely distorted.

4. In contrast, cat SAII receptors maintained about 70% of their control response after 20 min of neomycin infusion. The interspike interval histogram showed an orderly shift towards longer intervals maintaining its normal shape.

5. In rats, intradermal microinfusion of neomycin (30 μg/min) through a glass micropipette into the immediate vicinity of the receptor under investigation resulted in severe transient suppression of SAI receptor responses to about 10% of the control level. Receptor responses recovered almost completely about 1 h after the end of neomycin application.

6. It is concluded that the observed differences between the two types of slowly adapting mechanoreceptors are consistent with the hypothesis that the SAI receptor functions as a secondary sensory receptor, with a synaptic link between the Merkel cell and the primary afferent neurone.

INTRODUCTION

Over a wide range of species slowly adapting low-threshold mechanoreception in the skin is mediated through two different designs of sensory receptors. Perpendicular

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mechanical cutaneous stimulation is detected preferentially by the presumed secondary sensory type Merkel cell or SAI receptor, positioned at the epidermo-dermal border (Iggo & Muir, 1969). Cutaneous stretch, in contrast, is readily monitored by the primary sensory type Ruffini ending or SAII in the deeper dermal tissues (Chambers, Andres, v. Duehring & Iggo, 1972). The modes of transduction are not clearly understood for either type of sensory receptor. Structurally, both receptor types are closely interwoven with the tough tissues of the skin (see: Iggo & Andres, 1982; Munger & Ide, 1988). In their fully developed state they are therefore not easily accessible to electrophysiological investigation at the cellular level.

There is extensive similarity in the structural arrangement between hair cells in the inner ear and SAI receptors. Hair cell receptors are secondary mechanoreceptors. Techniques have been developed to isolate single hair cell receptor cells for electrophysiological investigation (Ohmori, 1984). In such experiments, amino-glycosides were found to decrease mechano-electrical currents in a reversible fashion (Hudspeth, 1982; Ohmori, 1985). At the neuromuscular junction amino-glycosides are known to suppress synaptic transmission (Prado, Corrado & Marseillan, 1978).

The present experiments were performed in order to compare the known effects of neomycin on these and other excitable tissues with its action on SAI receptors. The special aim was to differentiate between a synaptic or non-synaptic functional arrangement in the SAI receptor, with the working hypothesis that there is a synaptic link between Merkel cells and primary afferent neurones. To further emphasize this aim, the study has been designed as a comparison of the responses of this mechanoreceptor with those of a mechanoreceptor known to be of the primary sensory type, the SAII receptor.

On the grounds of differential sensitivity to hypoxia between primary afferent nerves and Merkel cells, Findlater, Cooksey, Anand, Paintal & Iggo (1987) support the concept of synaptic transmission between Merkel cell and afferent neurone. In line with the organization of hair cell receptors in the inner ear, Merkel cells will function as mechano-electrical transducers in the synaptic model. In contrast Diamond, Mills & Mearow (1988) argue that Merkel cells must have a function other than transduction, because rat SAI receptors are still sensitive to mechanical stimulation following chemical destruction of the Merkel cells.

Slowly adapting type I and less frequently SAII receptors have been exposed experimentally to a wide range of pharmacological substances (Fjaellbrant & Iggo, 1961; Smith & Creech, 1967). The only pharmacological principle to emerge was that they were sensitive to organic and inorganic Ca^{2+} antagonists (Cooksey, Findlater & Iggo, 1984; Pacitti & Findlater, 1988). In preliminary studies it has also been shown that mechano-electrical transduction is inhibited by acute exposure to neomycin in SAI and to a lesser extent SAII receptors (Baumann, Hamann & Leung, 1986; Baumann, Hamann & Leung, 1987).

The present experiments are a detailed analysis of the action of neomycin on both receptors, with the aim of testing the hypothesis that SAI receptors are secondary sensory receptors.

METHODS

Experiments on cats

Preparation of experimental animals. Cats of either sex (1·5–2·5 kg body weight) were anaesthetized initially with thiopental sodium (60 mg/kg I.P.) and subsequently 1·5% pentobarbitone sodium I.V. as required. The animals were placed on their back on a thermostatically controlled electric blanket keeping the rectal temperature at about 37 °C. The cats were breathing spontaneously through a cannula inserted into the trachea. Catheters were placed into the left carotid artery for monitoring of arterial blood pressure (mean blood pressure was above 80 mmHg throughout the experiments) and into a jugular vein for venous access. Using a microscope, a small catheter was inserted into the right medial circumflex femoris artery in a retrograde fashion, to allow infusion of fluids into the femoral artery without substantial change in femoral blood flow. The right hindlimb was kept in a half-extended position with the paw firmly fixed with an instrument clamp.

A paraffin pool was made from skin flaps at the medial part of the right thigh for dissection of fine nerve strands from the saphenous nerve. The nerve strands were placed on Ag–AgCl hook electrodes and single-unit recordings were made from afferent fibres supplying slowly adapting mechanoreceptors in the skin of the anterior aspect of the lower leg. Type I units were identified by their characteristic discharge in response to maintained mechanical stimulation of discrete spots in the skin only (Iggo & Muir, 1969; Horch, Whitehorn & Burgess, 1974) whereas type II units could also be excited by dermal stretch (Chambers *et al.* 1972).

Mechanical stimulation and data recording. Standard mechanical stimuli were applied every 30 s through a Perspex stylus (probe diameter 1 mm, spherical tip) attached to a force transducer (Statham UC2) driven by an electromechanical transducer (Bruel & Kjaer No. 4810). Each stimulus comprised a 200 ms ramp followed by a 2000 ms plateau phase (Fig. 1*A* and *B*) during which the force was maintained at 20 mN using a feedback control unit (developed in conjunction with the Electronics Department of the Chinese University of Hong Kong). This force of stimulation was found to give a near-maximal number of nerve impulses in both types of receptors (Baumann, Cheng-Chew, Hamann & Leung, 1984). Between stimuli a contact force of 0·5 mN was maintained. Displacements of the probe were continuously recorded by a displacement transducer (Sangamo Model DF5, Schlumberger).

Original recordings of forces, displacements and nervous responses were made with an FM tape-recorder (Hewlett–Packard 3964A) and displayed simultaneously on an oscilloscope (Tektronix 5103N) and chart recorder (Graphtec mark VII, 4 channels: blood pressure, force, displacement, and cumulative count of nerve impulses per stimulus). Data were digitized using an A/D converter (ISAAC) in conjunction with a 6502-based microcomputer. Following completion of the acute experiments tapes were played back for spike-train analysis using a bin width of 1 ms for responses during mechanical stimulation and 4 ms for on-going activity between stimuli.

Experimental protocol. Normal saline was infused into the femoral artery at a rate of 0·025 ml/min throughout the experiments except during periods of neomycin infusion lasting up to 20 min when the same volume delivered 2·5 mg/min neomycin as sulphate (Mycifradin^{R}, Upjohn) dissolved in distilled water.

Local tissue concentrations of neomycin were dependent on femoral blood flow. In analogy to data obtained in dogs (Mason & Ledsome, 1974) the femoral blood flow of the cats in this study is assumed to be in the range of 5–10 ml/min. The resulting transient plasma concentrations of neomycin reaching the leg are estimated to be between 250 and 500 mg/l ($4–8 \times 10^{-4}$ M) and are likely to equilibrate quickly with the interstitial fluid.

Initial periods of saline infusion lasted between 30 and 60 min to ensure steady-state responses. A small dimple developed under the stimulating probe within the first 10 min of stimulation which could be measured as 'residual indentation' at 0·5 mN contact force. Depending on the compliance of the skin and underlying tissue at the position of the receptor under investigation, residual indentations after the first twenty stimuli were between 0·1 and 0·5 mm and did not increase by more than 0·1 mm over the following 100 stimuli. 'Maximal indentations' of the probe required to maintain a stimulation force of 20 mN for a 2 s plateau phase were between 0·7 and 3·0 mm. Thus, the resulting stroke amplitudes of the stimulating probe (difference between maximal and residual indentation) were between 0·6 and 2·5 mm and did not change in their respective values throughout the experiments.

At the end of an experiment, Methylene Blue was injected to stain the area of skin within the range of the close arterial infusion. Only receptors well inside this area were included in the evaluation. Finally, the animals were painlessly killed with an overdose of pentobarbitone sodium.

Evaluation. A total of nineteen SAI receptors were examined with different infusion times of neomycin of 5 ($n = 6$), 10 ($n = 5$) and 20 min ($n = 8$). The results were compared with those

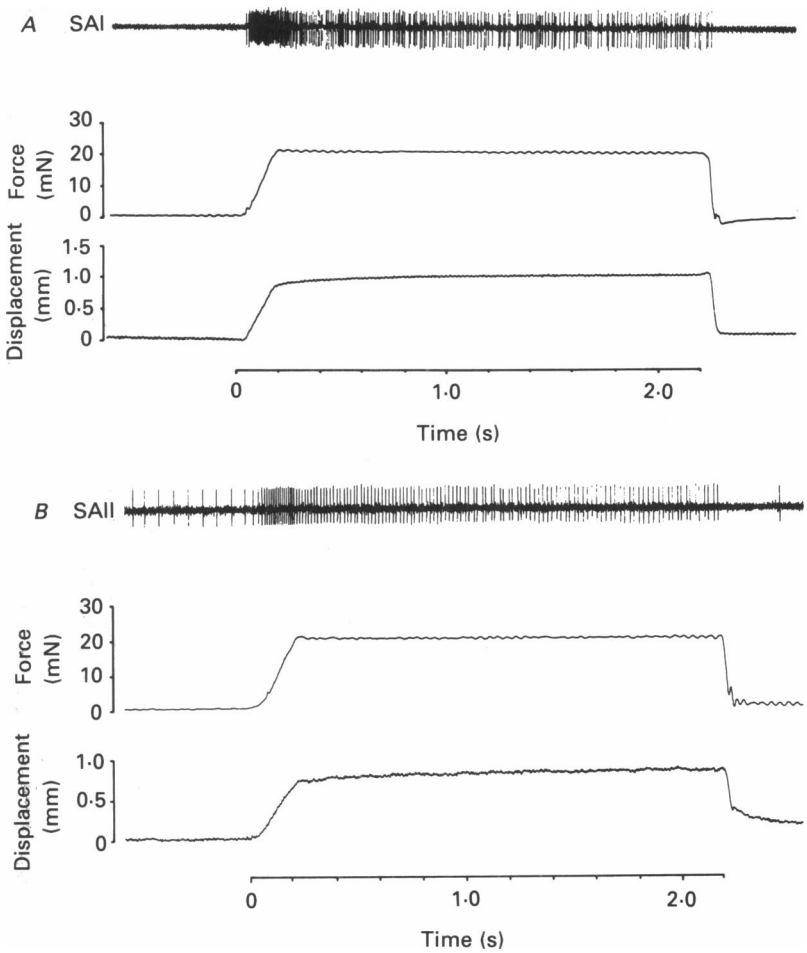

Fig. 1. Original recordings of responses of a cat SAI receptor (*A*) and a cat SAII receptor (*B*) to a standard mechanical stimulus of 20 mN constant force. Top traces show the action potentials recorded from a single afferent unit, middle traces show the force profile and bottom traces the displacement of the stimulation probe (200 ms ramp followed by a 2000 ms plateau phase).

obtained from six SAII receptors all of which had been subjected to 20 min of neomycin infusion. Statistical presentation of the data is given as mean values ± S.E.M. Student's t test was employed for comparison between the two groups of receptors. For graphical presentation and comparison of the two types of receptors subjected to infusion of neomycin lasting 20 min (listed in Table 1), three receptors were selected from each group because their absolute responses were of comparable magnitude throughout the experiments.

Modification of the methods for the experiments on rats

Male Sprague–Dawley rats (320–380 g) were anaesthetized with urethane (20% w/v; 6 ml/kg I.P.). The spontaneously breathing animals were placed on a thermostatically controlled electric

TABLE 1. Responses (impulses/s) of eight SAI and six SAII receptors in cats to standard mechanical stimuli of 20 mN constant force during the dynamic phase (Dyn, 0–300 ms), static phase (Stat, 300–2200 ms) and for the total duration (Total, 0–2200 ms). For SAII receptors the on-going discharge during the intervals between stimuli is given in the column headed 'Ong'. Listed are the control responses during the steady state before neomycin infusion, responses during maximum suppression shortly after 20 min of neomycin infusion of 2·5 mg/min (neomycin 50 mg) and after a recovery period of about 1 h (if available)

Cat SAI receptors

Cat No.	Control			Neomycin (50 mg)			Recovery		
	Dyn	Stat	Total	Dyn	Stat	Total	Dyn	Stat	Total
1	224	83	101	86	18	27	116	34	45
2	142	89	95	63	20	25	66	24	30
3	182	114	122	83	42	47	76	41	45
4	102	64	68	46	25	27	50	28	30
5	92	34	41	23	6	8	30	7	10
6	201	76	92	53	15	20	83	27	34
8	92	51	56	30	11	14	33	16	18
7	76	81	79	40	38	38	—	—	—
Mean	139	74	82	53	22	26	65	25	30
S.E.M.	19	8	9	8	4	4	11	4	5

Cat SAII receptors

Cat No.	Control				Neomycin (50 mg)				Recovery			
	Ong	Dyn	Stat	Total	Ong	Dyn	Stat	Total	Ong	Dyn	Stat	Total
1	11	46	30	32	5	30	20	21	11	33	22	23
2	15	46	31	32	6	33	21	23	13	43	25	27
3	18	46	37	37	13	30	24	25	14	33	27	27
4	14	129	73	79	6	112	48	56	15	119	55	63
5	24	63	40	42	19	50	30	32	—	—	—	—
6	16	76	49	52	6	69	30	35	—	—	—	—
Mean	16	68	43	45	9	54	29	32	13	57	32	35
S.E.M.	2	12	6	8	2	12	4	5	1	18	7	8

blanket and the arterial blood pressure was monitored through a catheter in one carotid artery. A paraffin pool was formed from skin flaps over the lower back and single-unit recordings were made from afferent fibres in cutaneous nerves supplying slowly adapting mechanoreceptors in the gluteal skin using the same recording technique as described for the cat experiments.

After identification of a slowly adapting mechanoreceptor the tip of a glass micropipette was inserted into the skin nearby with the tip at a distance of about 2 mm from the receptor. After obtaining steady responses to mechanical stimuli of 15 mN constant force with the same time parameters as described in detail for the cat experiments, neomycin solution of 100 mg/ml (as sulphate; Mycifradin^{R}) was infused at a rate of 0·3 μl/min (delivering 30 μg neomycin/min) for 10 min. In control studies saline was infused at the same rate.

In order to reduce the risk of breakage of the micropipette tips a plateau force of 15 mN rather than 20 mN was chosen, resulting in a reduced displacement of the skin surface.

RESULTS

Cat slowly adapting type I receptors.

All receptors included in this study reached maintained response levels within 30 min after starting mechanical stimulation with standard stimuli of 20 mN

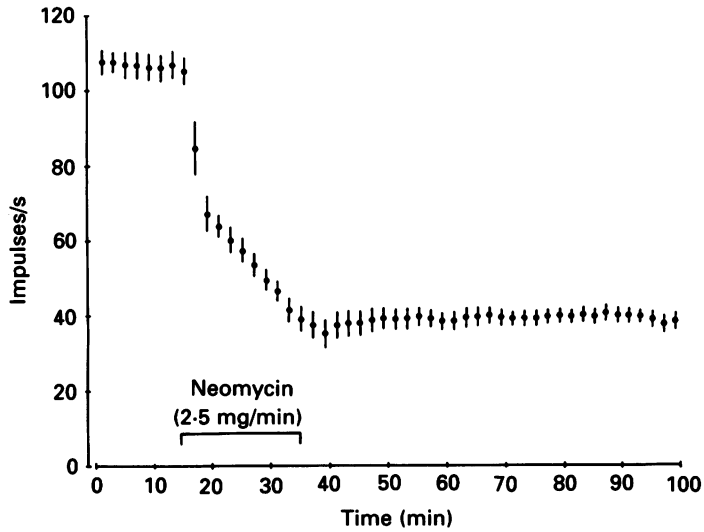

Fig. 2. Mean number of impulses per stimulus (in impluses/s) from three cat SAI receptors with similar discharge rates (the first three receptors of Table 1) in the time course of a typical experiment. From minute 1 to 14 saline was infused at a rate of 0·025 ml/min into the femoral blood stream followed by 2·5 mg neomycin/min given in the same volume from minute 15 to 34. For the rest of the experiment the infusion was changed back to saline.

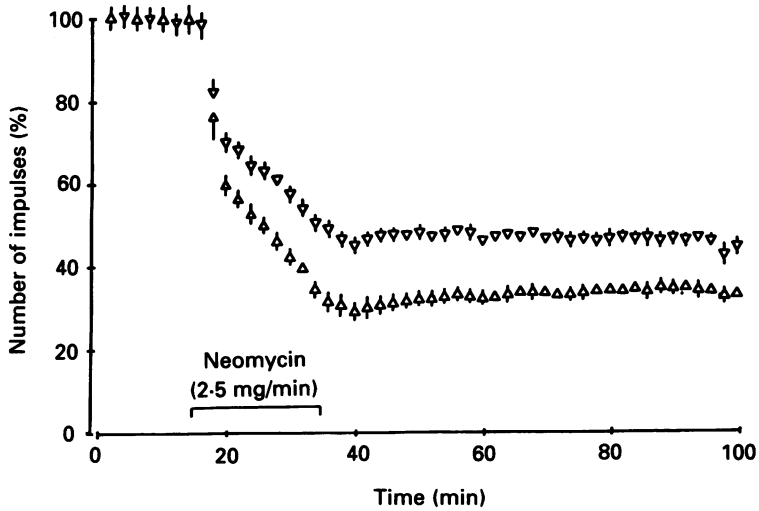

Fig. 3. Dynamic (▽) and static (△) responses of the same cat SAI receptors as in Fig. 2 plotted as percentage of their respective control values during the last 10 min before neomycin infusion throughout the time course of the experiments.

constant force every 30 s. In the eight SAI receptors subjected to 20 min of neomycin infusion (Table 1) the average discharge rate during the control period was 82 ± 9 impulses/s. This level of response remained essentially stable during periods of prolonged saline infusion. During the first 300 ms of individual stimuli the average firing rate (dynamic response) was 139 ± 19 impulses/s and adapted to an average of 74 ± 8 impulses/s during the 2 s plateau phase.

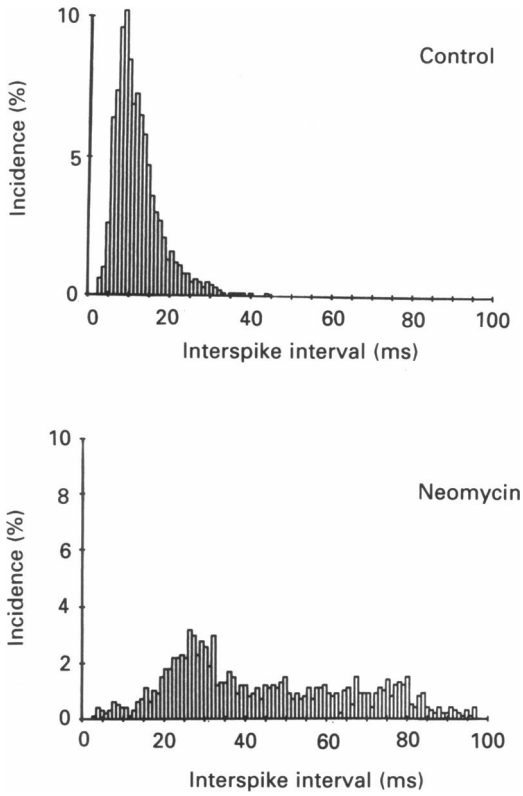

Fig. 4. Interspike interval histograms of the same three cat SAI receptors as in Figs 2 and 3 during the static phases of mechanical stimulation (1200–2200 ms) for ten control responses (coefficient of variation, c.v. = 0·52) and ten responses shortly after the end of 20 min neomycin infusion (c.v. = 0·57).

Close arterial infusion of 2·5 mg neomycin/min was started between 30 and 60 min after the onset of stimulation when both the mechanical parameters of stimulation and the response levels had reached stable levels. No significant changes could be observed in residual indentation at contact force nor in maximal indentation required to maintain the 20 mN constant force of stimulation. In contrast, nervous responses dropped sharply immediately after neomycin had reached the femoral blood stream. Within 5 min of the start of infusion responsiveness declined to nearly half of the original level reaching a minimum at about 30% of the control level (26 ± 4 impulses/s) at the end of the 20 min infusion period (Fig. 2). Generally, dynamic responses were found to be relatively less affected by neomycin than static

responses (Fig. 3). No appreciable recovery in responsiveness of the receptors could be observed during 1 h of saline infusion following neomycin.

Interspike interval histograms of the discharge during the last 1000 ms of the plateau phase showed the typical Poisson-like distribution with the highest incidence of intervals in the bins between 6 and 10 ms. Immediately following the end of the neomycin infusion, interspike interval histograms were severely distorted (Fig. 4). The range of intervals observed was broadened to between 1 and 100 ms.

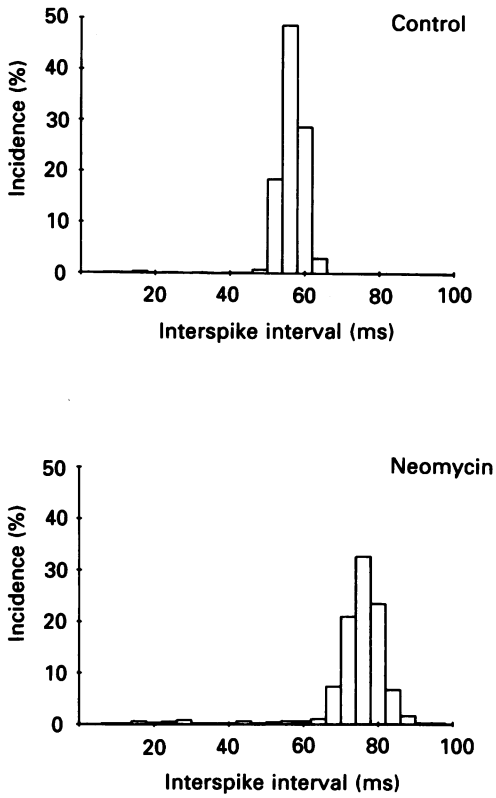

Fig. 5. Interspike interval histograms of the on-going discharge of a cat SAII receptor (No. 3 in Table 1) during 0·5 mN contact force between stimuli (18–28 s after the start of the previous stimulus). The upper part shows again the control situation (c.v. = 0·095) while the lower part was obtained shortly after the end of 20 min infusion of neomycin (c.v. = 0·158).

Almost identical rates of decrease in responsiveness were obtained for different periods of neomycin infusion at the same rate of 2·5 mg/min levelling off shortly after changing back to saline infusion. Numbers of impulses were reduced to $62 \pm 7\%$ ($n = 6$) after 5 min (12·5 mg neomycin) and $45 \pm 5\%$ ($n = 5$) after 10 min (25 mg neomycin) of their respective control levels. Distortion of the interspike interval histograms was less severe after these shorter periods of infusions.

Cat slowly adapting type II receptors

On-going activity in slowly adapting type II receptors averaged at 16 ± 2 impulses/s, and there was a relatively low discharge rate in response to the same

standard mechanical stimuli of 20 mN constant force. The average value during the control period at the beginning of experiments was 45 ± 8 impulses/s. Dynamic and static responses were 68 ± 12 and 43 ± 6 impulses/s respectively (Table 1).

Interspike interval histograms during on-going activity of individual receptors

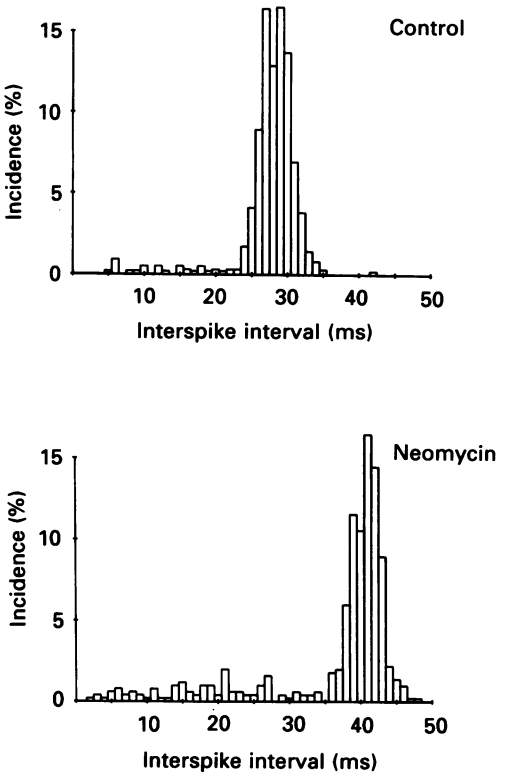

Fig. 6. Interspike interval histograms of ten static responses (1200–2200 ms) each during the control period (c.v. = 0·34) and shortly after the end of 20 min neomycin infusion (c.v. = 0·38) for the same cat SAII receptor as in Fig. 5.

showed typically narrow distributions around the interval with the highest incidence. In the example shown in Fig. 5 (SAII receptor No. 3 in Table 1), more than 90% of the intervals fall in the range between 50 and 60 ms. Correspondingly, the coefficient of variance was below 0·1. Interspike interval histograms of static responses (during the last 1000 ms of individual stimuli) showed a similarly narrow distribution around the mean interval (Fig. 6). The coefficient of variance was found to be in the range of 0·30–0·35 indicating incomplete adaptation during the time interval of 1200–2200 ms after the beginning of individual stimuli (Chambers *et al.* 1972).

Infusion of neomycin (2·5 mg/min) into the femoral artery was started after a control period of 30–40 min at a time when the mechanical parameters of stimulation as well as nervous responses had reached stable conditions. Both residual and maximal indentation did not change significantly from the previous levels after switching from saline to neomycin infusion.

On-going activity of the type II receptors fell sharply within minutes after the start of neomycin infusion to on average about half of its control level at the end of

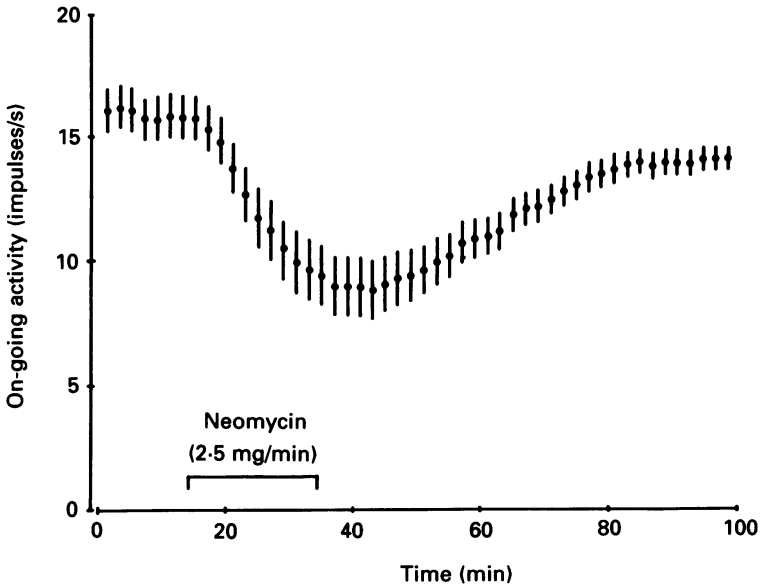

Fig. 7. On-going activity as mean discharge rate in impulses/s of three cat SAII receptors (the first three type II receptors in Table 1) during the typical time course of experiments. From minute 15 to 34 neomycin was infused into the femoral blood stream at a rate of 2·5 mg/min. Beforehand and afterwards saline was infused at the same volume of 0·025 ml/min.

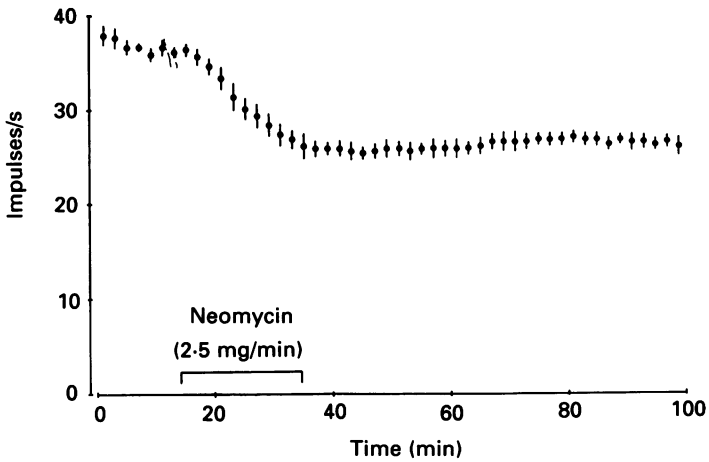

Fig. 8. Mean number of impulses in responses to standard mechanical stimuli (in impulses/s) for the same three cat SAII receptors with the same experimental protocol as in Fig. 7.

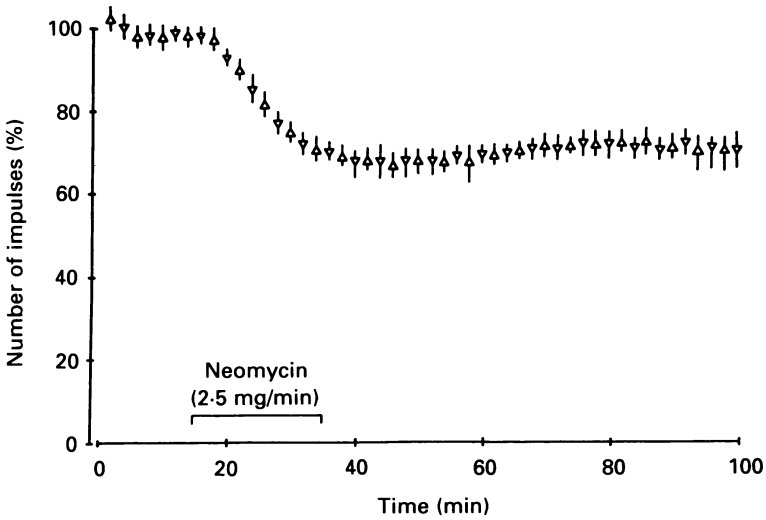

Fig. 9. Dynamic (▽) and static (△) responses to standard mechanical stimuli of the same cat SAII receptors as in Fig. 8 plotted as a percentage of their respective control values during the last 10 min before neomycin infusion throughout the typical time course of the experiments.

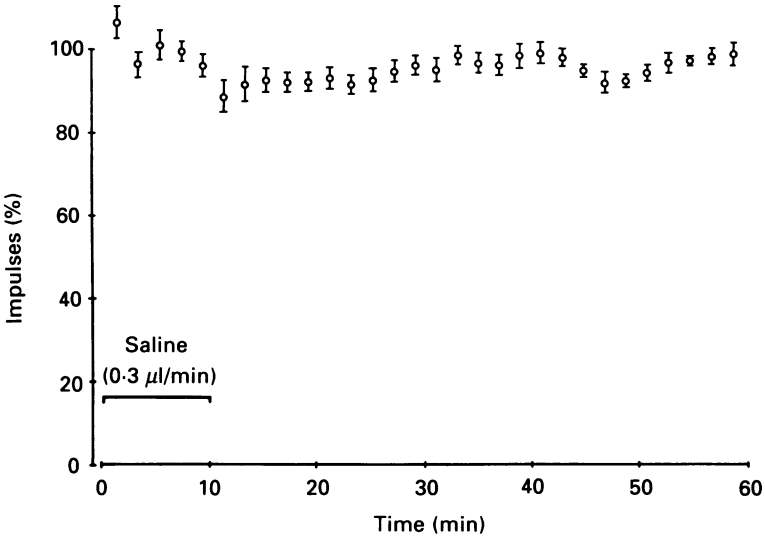

Fig. 10. Mean number of impulses in response to 15 mN constant force stimuli as a percentage of the control responses during the last 10 min before intradermal saline microinfusion for two rat SAI receptors. Saline was infused through a glass micropipette into the immediate vicinity of the receptor at a rate of 0·3 μl/min from minute 1 to 10. No significant effect on receptor responses could be observed.

the 20 min period of neomycin infusion (9 ± 2 impulses/s; Fig. 7). Recovery of the on-going activity during the following hour of saline infusion was nearly complete (13 ± 1 impulses/s). In contrast, responses to standard mechanical stimuli were affected less, falling gradually to about 70% of the control level during the period of neomycin infusion (Fig. 8). Dynamic and static responses were equally affected without any difference in their relative responsiveness as compared with the control levels (Fig. 9). During the 1 h period of saline infusion following neomycin there was only minimal recovery of the nervous activity in response to mechanical stimulation.

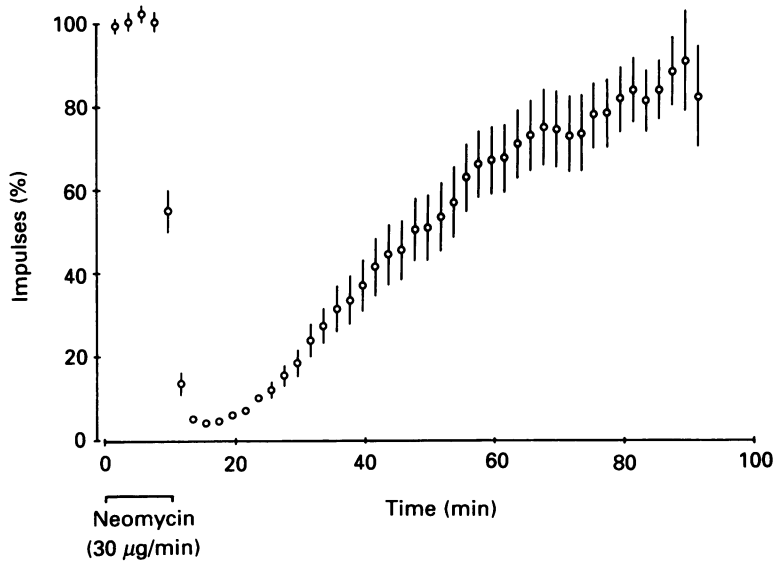

Fig. 11. Mean number of impulses per stimulus from five rat SAI receptors expressed as a percentage of the control responses during the last 10 min before intradermal microinfusion of neomycin. From minute 1 to 10 neomycin was infused at a rate of 30 μg/min into the immediate vicinity of the receptors.

Interspike interval histograms both for on-going and stimulated activity showed shifts of the mean interval to the right with slight broadening of the distributions and small increases in the coefficients of variance. However, the principal shape of the distributions reflecting the regular discharge pattern of the type II receptors did not change (Figs 5 and 6).

Rat slowly adapting type I

During control experiments with microinfusion of saline (two units) into the immediate vicinity of the receptors responsiveness stayed at ⩾ 90% of the initial level for more than 1 h (Fig. 10). In contrast, immediately after starting the intradermal microinfusion of neomycin the responsiveness of all ten SAI receptors examined declined sharply. The time course and extent of the depression appeared to depend on how close the micropipette was positioned to the receptor. In five units a similar pattern of suppression was observed reaching a minimum of less than 10% of the control response about 5 min after the end of the 10 min infusion period

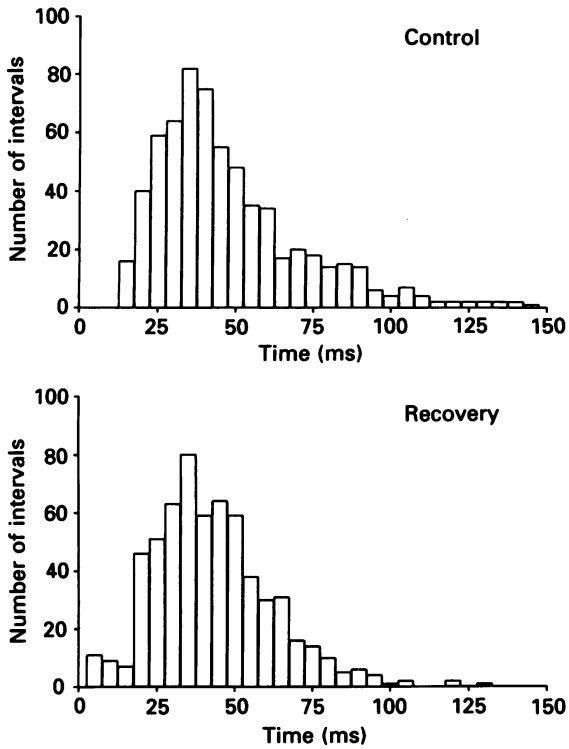

Fig. 12. Interspike interval histograms of static responses of the same five rat SAI receptors as in Fig. 11 during the control period before neomycin microinfusion (upper graph) and at the end of about 1 h recovery period after neomycin microinfusion (lower part).

(Fig. 11). Over the following hour receptor responses returned gradually to about 90% of the control level.

Figure 12 shows the interspike interval histogram of the static responses (1200–2200 ms) of these receptors during the control period before microinfusion of neomycin compared with that at the end of the recovery period of 1 h. The histograms are nearly identical demonstrating almost complete recovery under these experimental conditions.

Rat slowly adapting type II receptors

Slowly adapting type II receptors were very difficult to find in the gluteal region of the rat skin. Thus, only one receptor could be examined in this study: microinfusion of neomycin had hardly any effect on the responses recorded from this receptor.

DISCUSSION

Differences in the responses of slowly adapting type I and II receptors

In the present results a clear differentiation was achieved between SAI and SAII cutaneous mechanoreceptors. All SAI receptors lacked on-going activity and responded to maintained perpendicular stimulation of discrete spots in the skin with a slowly adapting discharge (Iggo & Muir, 1969). In contrast SAII receptors presented with regular on-going nervous activity. A coefficient of variance smaller than 0·1 in the steady state of on-going activity unambiguously identified these receptors as of the SAII type (Chambers *et al.* 1972).

It was surprising to note the scarcity of SAII receptors in the gluteal region of the rat. In spite of extensive efforts, only one SAII receptor was found. This apparent difference in species may, however, depend on a difference in topography. In the cat fine nerve strands were dissected from the saphenous nerve, supplying skin in the lower leg which is more tightly moulded to the features of the underlying tissue than is the case in the gluteal region. In very loose skin areas conditions are less likely to occur where SAII receptors could be excited, and such receptors might not serve a useful purpose. In their study on the distribution of myelinated afferent skin units in the saphenous nerve of the rat Lynn & Carpenter (1982) found two SAI and one SAII unit in a sample of 112 A-fibres. If this finding is representative, there should be more type II units in the rat saphenous nerve.

Close arterial infusion of neomycin caused profound suppression of responsiveness in SAI and to a lesser extent in SAII receptors (compare Figs 2 and 8). Immediately following a 20 min close arterial infusion of neomycin the depression of responsiveness lasted more than 1 h without significant recovery. Similar time courses were observed for both types of receptors. It appears that the initial high local concentration of neomycin sensitized the receptor mechanism to the action of the much lower levels prevailing after distribution of the infused neomycin in the extracellular space. In previous work (Baumann, Hamann & Leung, 1988) equal amounts of neomycin injected close arterially or intravenously did not cause the same degree of suppression. Furthermore, transient high local concentrations of neomycin are not followed by irreversible effects, as has been shown in the experiments using intradermal infusion (Figs 11 and 12).

In the present investigation a more detailed analysis was undertaken into dynamic and static components of receptor discharge. In SAI receptors neomcyin had a proportionately stronger effect during the plateau phase of stimulation. In contrast, SAII responses during dynamic and plateau phases of stimulation were affected to precisely the same degree (Fig. 9). There was no differentially enhanced sensitivity during the plateau phase. However, the on-going activity of SAII receptors was relatively more susceptible to the action of neomycin than responses during dynamic or plateau phases of stimulation (Figs 7 and 8).

It was interesting to compare the distributions of the interspike intervals between the two types of receptor. The last second of the plateau phase of individual mechanical stimuli, i.e. between 1200 and 2200 ms after the beginning of the stimulus, was chosen for analysis of interval distribution. At this time the responses

were close to steady state. Full adaptation of both types of slowly adapting receptors requires more than 10 s (Chambers *et al.* 1972; Horch *et al.* 1974). Thus, insufficient time would have been available for recovery of receptor responsiveness before application of the next stimulus in the present pattern of repetitive mechanical stimulation every 30 s.

In SAII receptors the regularity of the receptor discharge was maintained under the action of neomycin with only a slight increase in the coefficient of variance, e.g. from 0·34 to 0·38 (see Fig. 6). The narrow band of frequently occurring intervals was shifted towards the longer intervals on the right. This indicates a proportional reduction in effectiveness of mechano-electrical transduction. In contrast, the discharge pattern of SAI receptors is affected in a completely different way by the infusion of neomycin. In control recordings, interval histograms resembled a Poisson distribution as described by Iggo & Muir (1969). After neomycin infusion responses were still of the slowly adapting type with higher frequencies during the dynamic phase of stimulation. Interspike intervals of just a few milliseconds were still observed even at mean discharge frequencies of only about 20 impulses/s. Sequences of high-frequency discharge could thus still be conducted during the acute action of neomycin. This observation serves as evidence that the present findings are not caused by conduction failure in afferent nerve fibres. The most striking experimental finding during the infusion of neomycin was the transformation of the Poisson-like distribution of intervals to a widely distributed spectrum. There was no orderly shift of the control pattern towards larger intervals as was the case in the SAII receptors. This distortion was also observed in the experiments with intradermal microinfusion performed in rats. In these experiments neomycin could be largely removed from the receptor over about 1 h after termination of the microinfusion resulting in almost complete recovery of receptor discharge and distribution of spike intervals (Figs 11 and 12).

Mode of action of neomycin

In many aspects the present results are consistent with the action of neomycin as a Ca^{2+} antagonist. Organic as well as inorganic Ca^{2+} antagonists strongly suppress the excitability of mammalian SAI receptors (Cooksey *et al.* 1984; Pacitti & Findlater, 1988). In isolated hair cells from the inner ear, neomycin reversibly decreases the mechanosensitive receptor current (Hudspeth & Kroese, 1983; Ohmori, 1984). Hudspeth (1982) was able to produce partial to complete blockage of mechano-electrical transduction in hair cells by topical application of neomycin around the hair bundle. In terms of stimulus–response characteristics a decrease in mechanosensitive current can be expected to result in a shift towards lower discharge frequencies, but maintaining the overall pattern, as observed in SAII receptors. Physiologically, decreased mechanosensitive receptor currents may be caused by reducing the strength of natural stimulation. The resulting lower discharge rate in the afferent nerve fibre still shows the characteristic Poisson-like distribution.

In the light of this finding and the present comparative observations on SAII receptors, it is difficult to explain the sensory suppressive effect of neomycin on SAI receptors solely on the basis of reduced mechanosensitive receptor currents. But the

present findings would become easily explicable assuming an additional inhibitory effect on processes linking stimulus transduction and spike generation, with a synaptic mechanism between Merkel cells and nerve terminals being a likely target. Chemical synaptic transmission has been shown to be severely impaired by aminoglycosides at the neuromuscular junction and central synapses (Fiekers, 1983; Tolliver & Warnick, 1987; Tsai, 1987). The main mechanism of this inhibition is a Ca^{2+}-competitive suppression of presynaptic release of a transmitter substance (Wright & Collier, 1977). In order to achieve comparable postsynaptic reduction in excitability much higher concentrations of aminoglycoside are needed.

In the present work on mechanoreceptors in the cat doses of neomycin were used causing a significant but not complete suppression of responsiveness. In these experiments SAI receptors were found to be much more susceptible to the inhibitory effect of neomycin than SAII receptors, where the mechano-electrical transducer is located in terminal branches of the primary afferent neurone. This differential susceptibility between SAI and SAII receptors to neomycin is consistent with and, in line with the above findings on other synapses, can be explained best by the presence of a chemical synapse in SAI receptors.

Synaptic link between Merkel cell and afferent nerve fibre ?

Recently, Diamond and co-workers suggested that Merkel cells have functions other than mechano-electrical transduction (Diamond *et al.* 1988). Slowly adapting type I receptors where the Merkel cells had been destroyed with the fluorescent substance quinacrine were still found to be sensitive to mechanical stimulation. Merkel cell–neurite complexes are tightly structured. Destruction of one component will not leave the other part unaffected. It is known that regenerating nerve fibres are mechanosensitive (Brown & Iggo, 1963). Thus, quantitative and not qualitative equality of stimulus–response properties between control and test populations of receptors needs to be demonstrated, in order for this experiment to be conclusive.

Finally Cooper & Nurse (1986) succeeded with patch-clamp recordings from Merkel cells that had been removed from a follicle of a sinus hair by digestion. To test mechanosensitivity they applied small pressure pulses of perfusion fluid. Using this procedure the authors did not elicit any mechanosensitive membrane currents. In analogy to hair cells this mode of stimulation is equivalent to stimulation of the cell body and not to bending of the cilia. Merkel cells possess specialized processes pointing into spaces between keratinocytes. Assuming that Merkel cells are transducer cells, it is likely that, as in hair cells, shear force acting on these processes is the adequate stimulus for mechano-electrical transduction.

In conclusion, the present experiments have shown several profound differences in the inhibition of mechano-electrical transduction by neomycin between cutaneous mechanoreceptors of the primary sensory, the SAII, and presumed secondary sensory, the SAI, types. The differences are consistent with and strongly supportive of the hypothesis that the SAI receptor is of the secondary sensory type, with a synaptic link between Merkel cell and primary afferent neurone.

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