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CHEMICAL RESPONSES OF POLYMODAL RECEPTORS OF THE SCROTAL CONTENTS IN DOGS

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SUMMARY

1. Chemical responses of polymodal receptors of the superior spermatic nerve were studied by single unit recording in dogs. The responses were similar to those found in the muscular polymodal receptors of dogs.

2. Solutions of hypertonic NaCl, KCl and bradykinin caused responses in more than 90% of these polymodal units. The responses were related to the dose of these three chemical but discharge patterns differed substantially. Responses to Na citrate were very capricious, and those to acetylcholine and histamine were weak.

3. When bradykinin was applied repeatedly the responses of the subsequent trials decreased if the repetition interval was short (less than 15 min), but an increase of response was observed if the repetition interval was long (30–60 min).

4. Indomethacin 10 $\mu g.ml^{-1}$ suppressed or abolished the response to 1 $\mu g.ml^{-1}$ bradykinin without significant influence on the response to 60 $mmol.l^{-1}$ KCl at the same receptive site. Involvement of prostaglandins in the response of the polymodal units to bradykinin is suggested.

INTRODUCTION

The mechanical and thermal responses of the nociceptor component of the cutaneous polymodal population have been studied precisely (Bessou & Perl, 1969; Beitel & Dubner, 1976; Croze, Duclaux & Kenshalo, 1976; Kumazawa & Perl, 1977). However, responses to chemical stimulation have been reported only briefly: low frequency discharges to hydrochloric acid, sulphate and acetic acid (Bessou & Perl, 1969) and bursting discharges to histamine (van Hees & Gybels, 1972).

As reported in the previous paper (Kumazawa & Mizumura, 1980), polymodal receptors with axons in the superior spermatic nerve are thought to be located close to the surface of the testis and to be easily accessible to chemicals from the surface. Moreover, it might be advantageous that most of these receptors have multiple receptive sites.

In the present experiment responses of polymodal receptors to hypertonic NaCl, KCl, bradykinin, Na citrate, acetylcholine, and histamine were studied. These chemicals have also been tested on the muscular polymodal receptors (Kumazawa & Mizumura, 1976, 1977*a*). In this paper the incidence of response to these chemicals, dose–response relations of NaCl, KCl and bradykinin, tachyphylactic response to bradykinin, and suppression of bradykinin response by indomethacin are reported. The

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response characteristics to mechanical and thermal stimulation of the units included in this report were described in the previous paper (Kumazawa & Mizumura, 1980).

A preliminary report has appeared elsewhere (Kumazawa & Mizumura, 1977*b*).

METHODS

Operations and method of testing mechanical and thermal sensitivity are the same as in the previous paper (Kumazawa & Mizumura, 1980). Chemicals were applied on a receptive site with a cotton ball 1·5 mm in diameter soaked with the solutions. After application for one minute,

TABLE 1. Numbers of polymodal units responding to chemical stimuli. Units are entered as excited if at least one receptive site responded to a chemical. Solutions used: NaCl, 2·7–9%; KCl, 45–180 mmol.l^{-1}; bradykinin, 0·1–10 μg.ml^{-1}; Na citrate, 3–18%; acetylcholine, 0·1–10 μg.ml^{-1}; histamine, 1–10 mg.ml^{-1}. All units responded to at least one of the above, and also to mechanical and heat stimulation

Chemicals	No. excited	No. not excited	Total
NaCl	69	2	71
KCl	61	0	61
Bradykinin	83	6	89
Na citrate	37	7	44
Acetylcholine	7	7	14
Histamine	4	3	7
At least one of the above	93	0	93

the surface of the receptive site was washed with Ringer–Locke solution. Temperatures of the stimulating area and a cotton ball soaked with a solution were maintained at 33–35 °C by radiant heat. KCl solution was made by mixing isotonic KCl solution and Ringer–Locke solution. Concentrations of chemicals usually used were as follows: NaCl 4·5 and 9% (w/v), KCl 60 mmol.l^{-1}, Na citrate 6% (w/v), bradykinin 1 and 10 mg.ml^{-1}, acetylcholine 1 mg.ml^{-1}, histamine 1 mg.ml^{-1}. In limited cases, including those for examining dose–response relations, the following concentrations were also used: NaCl 1·8, 2·7, 3·6, 5·4 and 6·3% (w/v); KCl 15, 30, 45, 90, 150 and 180 mmol.l^{-1}; Na citrate 3, 9 and 18% (w/v); bradykinin 0·01, 0·05, 0·1, 0·3, 0·5 and 5 μg.ml^{-1}; acetylcholine 0·01, 0·1, 0·5 and 10 mg.ml^{-1}; histamine 10 mg.ml^{-1}.

Intervals between applications were usually 5–10 min. Ringer–Locke solution applied in the way described above caused no response in most cases, but it occasionally caused a slight increase of discharge at the onset of application in a few mechanically highly sensitive units.

Indomethacin 0·01 mol.l^{-1} was made by being dissolved with equimolar NaOH, thereafter the solution was diluted with Ringer–Locke solution resulting in a concentration of 10 μg.ml^{-1}.

RESULTS

Incidence of the response to various chemicals

The response to various chemical solutions was tested at one or several receptive sites of ninety-three units which responded to both mechanical and heat stimuli. All of these units responded to more than one kind of chemical solution listed in Table 1. In a few cases a solution that was effective at one receptive site did not cause responses at the other site of the same unit; presumably due to differences in access of solution between the receptive sites.

As shown in Table 1, the great majority of units responded to KCl, hypertonic

NaCl, and bradykinin, with a gradually decreasing incidence in that order. Sodium citrate caused response in 84 % of the tested units but the response was particularly irregular as is described later. Responses to acetylcholine and histamine were obtained in about half of the units tested but discharge rates were low and latencies long.

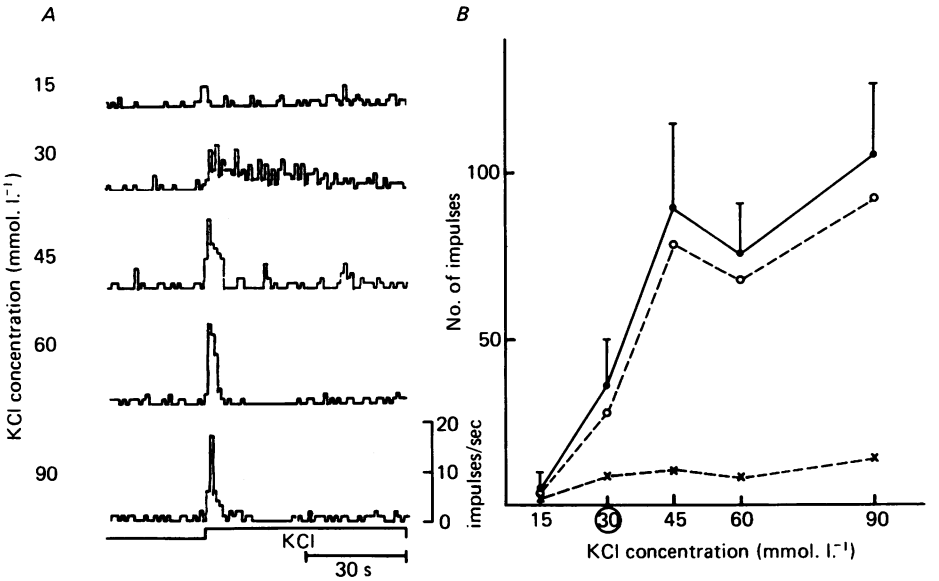

Fig. 1. Responses to KCl solutions of different concentrations. *A*, discharges of a polymodal unit to KCl solutions. Numbers of impulses during 1 s period are depicted as columns with a scale at the lower right. Concentrations used are shown at the left. Period of KCl application is designated with upward deflexion of the line at the bottom. Conduction velocity: 12·7 m.s^{-1}; receptive field: on the testis and epididymis. *B*, dose–response relationship. Numbers of units tested are seven for 15 mmol.l^{-1} and ten for the concentrations more than 30 mmol.l^{-1}. The lowest concentration which induced a significant increase in discharge rate ($P < 0{\cdot}05$) is designated by an encircled number. Filled circle: net increase of discharge during 60 s period of application; open circle: net increase of discharges during first 30 s periods of application; cross: net increase of discharges during last 30 s period of application; vertical bar: S.E. of mean. Note: most of the discharge increase is attributed to the first half.

Characteristic features of responses to various chemicals

Response to KCl. Most units started discharging within one second after the application of KCl solution (Fig. 1*A*, Table 2). In a few units a slight increase of discharges was observed at a concentration of 15 mmol.l^{-1} but a statistically significant ($P < 0{\cdot}05$, *t* test) response in ten units was only obtained above 30 mmol.l^{-1}. The total number of impulses during the period of KCl application increased in relation to the concentration of the solution (Fig. 1*B*). As shown in Fig. 1*A*, with solutions of lower concentrations discharges increased and decayed slowly, but with concentrations higher than about 45 mmol.l^{-1}, discharges increased and declined steeply and were followed by a complete or incomplete lull. This characteristic response pattern is exhibited by the preponderance of impulses during the first half of the stimulation, as shown by the open circle in Fig. 1*B*, and is also demon-

strated in Table 2 by a short 'time to peak' and large 'peak discharge rate' despite a small 'mean discharge rate'.

After the response with a silent period, mechanical response at the same receptive site was either abolished or highly diminished until the site was washed several times.

Table 2. Characteristics of responses of polymodal units to chemical stimuli. All values are means with standard error. Latency: time to the bin which showed an apparent increase of discharges. Peak discharge rate: the maximum impulse numbers during 1 s in 60 s application period in the units with an apparent peak or with peaks. Time-to-peak: time to the bin which had a maximum number of impulses. Mean discharge rate: mean impulse number during 60 s application period. Numbers of receptive sites tested are indicated in parentheses

	Latency (s)	Peak discharge rate (impulses)	Time to peak (s)	Mean discharge rate (impulses)
NaCl (4·5%)	4·0 ±1·0 (50)	12·6±1·5 (46)	21·2±2·4 (46)	4·3±0·5 (48)
KCl (60 mmol.l^{-1})	0·5 ±0·2 (51)	15·2±1·1 (48)	3·1±0·5 (48)	2·4±0·2 (48)
Bradykinin (10 μg.ml^{-1})	17·4±1·7 (61)	5·8±0·3 (51)	28·5±2·0 (51)	2·2±0·2 (61)
Sodium citrate (6%)	10·2±3·4 (24)	18·0±3·6 (16)	26·2±5·6 (16)	3·6±0·9 (22)

Response to NaCl. The solution of 1·8% NaCl was not effective, and 2·7% caused responses in two out of seven units. A statistically significant response was obtained above 3·6%. As shown in Fig. 2*A*, discharges increased and decreased irregularly and usually continued during the whole stimulating period with multiple peaks. A high mean-discharge rate in Table 2 and a relatively even distribution of impulses in both halves of the stimulating period, as shown by the open circle and cross in Fig. 2*B*, indicates this characteristic discharge pattern. The dose–response relationship, plotted from the results of seven polymodal units is shown in Fig. 2*B*.

Response to bradykinin. The discharge pattern in response to bradykinin was characterized by a slow and smooth increase and decline with a long latency (Fig. 3*A*). A larger number of impulses appeared in the latter half of the stimulating period. The dose–response curve (Fig. 3*B*) shows a statistically significant response above 0·1 μg.ml^{-1}, and the maximal number of impulses was evoked with 5 μg.ml^{-1}. Among responses to four chemicals listed in Table 2, response to 10 μg.ml of bradykinin, which is the supramaximal dose, shows the longest latency and time-to-peak and the smallest peak- and mean-discharge rates (Table 2). As the dose–response experiments were carried out in the order from lower to higher concentrations with a 5–10 min interval, possibly tachyphylaxis caused a reduction of the responses to higher concentrations tested later.

Response to Na citrate. Response to Na citrate was usually very capricious. This is shown by the highest standard errors in all items listed in Table 2. Fig. 4 shows an example of the responses of a unit to increasing concentrations of Na citrate; the response increased from 0·5 to 3%, but with 6% it almost disappeared and again with 9% a large one appeared with a long latency. Reproducibility of these responses was so poor that the dose–response relationship of Na citrate could not be studied.

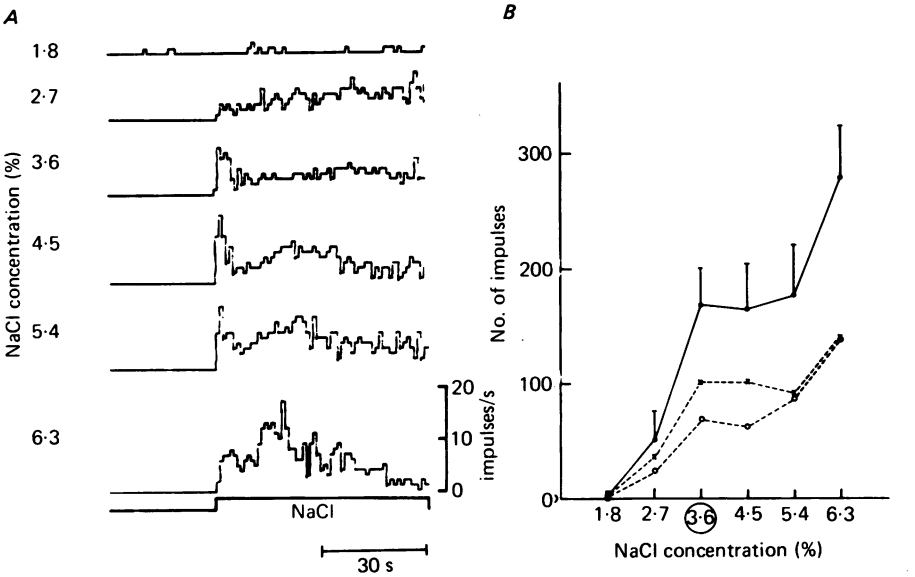

Fig. 2. Responses to hypertonic NaCl solutions of different concentrations. *A* discharges of a polymodal unit to NaCl solutions. Method of presentation is the same as in Fig. 1. Conduction velocity: 13·2 m.s^{-1}. Receptive field: on the testis. In this unit an increase in discharge appeared at concentrations more than 2·7%. *B* dose–response relationship. Number of units tested is seven for all concentrations. Symbols are the same as in Fig. 1. Note: both halves of stimulating period contribute equally to the increase of discharges.

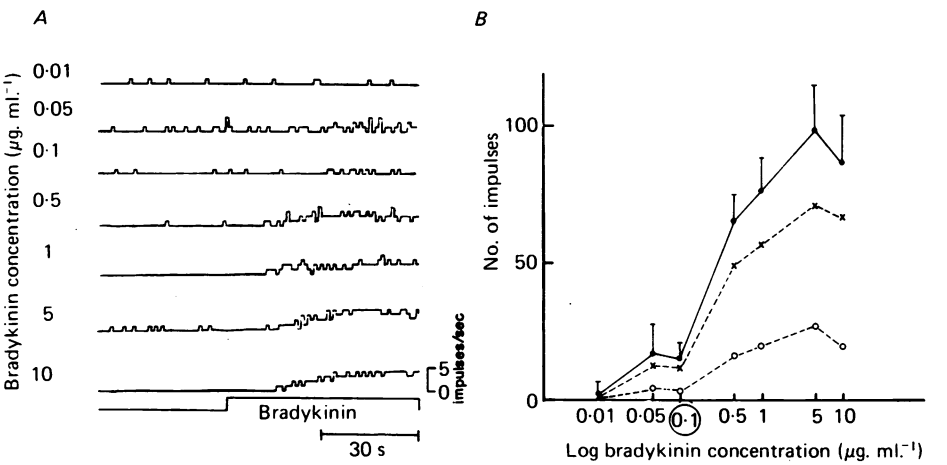

Fig. 3. Responses to bradykinin solutions of different concentrations. *A*, discharges of a polymodal unit to bradykinin solutions. The method of presentation is the same as in Fig. 1. Conduction velocity: 12·5 m.s^{-1}. Receptive field: on the testis. This unit showed an increase in discharge at concentrations more than 0·05 μg.ml^{-1} though increase at 0·1 μg.ml^{-1} is smaller than at 0·05 μg.ml^{-1}. Long latency and smooth increase of discharges are apparent. *B*, dose–response relationship. Numbers of units tested are six for 0·01 and 0·05 μg.ml^{-1}, and seven for more than 0·1 μg.ml^{-1}. Symbols are the same as in Fig. 1. Note: most of the increase of discharges is attributable to the latter half.

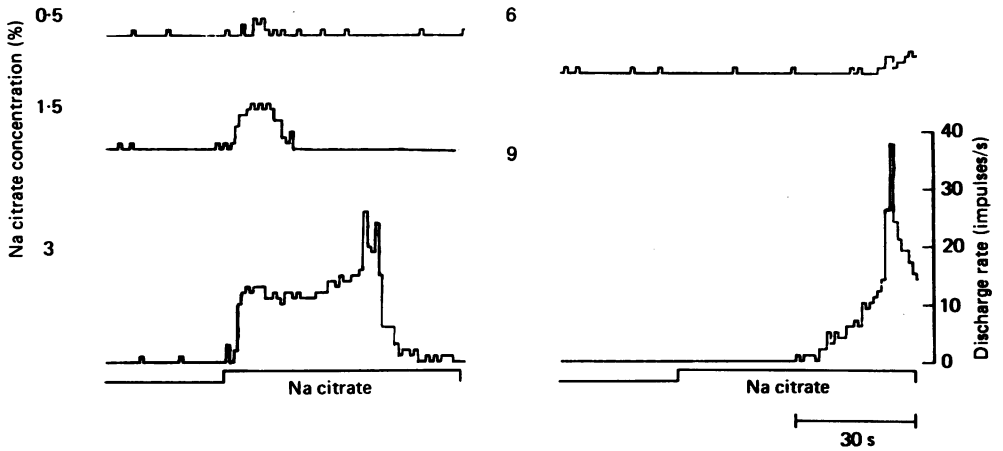

Fig. 4. Responses of a polymodal unit to increasing concentrations of Na citrate. The method of presentation is the same as in Fig. 1*A*. The same unit as in Fig. 1*A*. Note: no apparent dose–response relation.

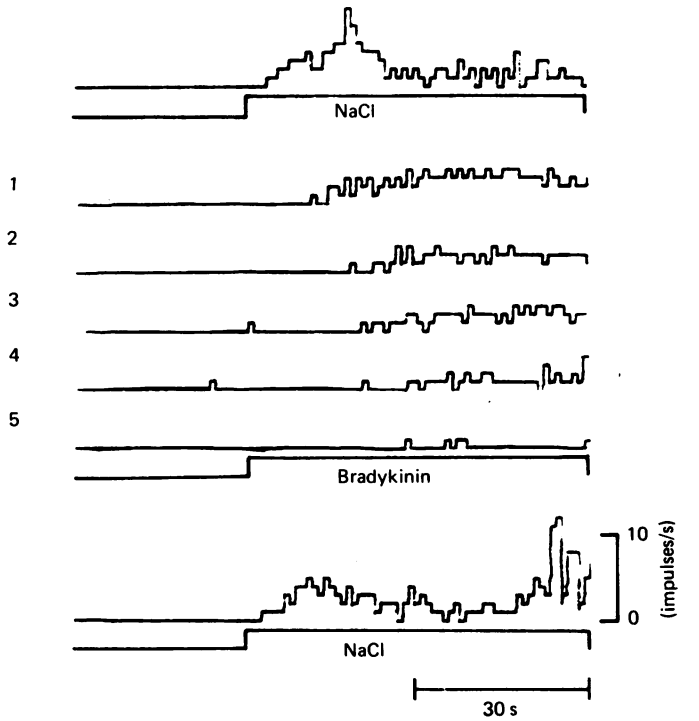

Fig. 5. Tachyphylaxis of a polymodal receptor to bradykinin. Bradykinin 10 $\mu g.ml^{-1}$ was applied five times, successive trials being interrupted by a 7 min interval. Before and after testing bradykinin, NaCl 4·5% was tested. Drawings of successive trials are orderly arranged from top to bottom. The method of presentation is the same as in Fig. 1*A*. Conduction velocity: not measured. Receptive field: on the testis, epididymis and spermatic cord. Note: marked reduction of bradykinin response on repetitive applications, contrasting with a preserved response to NaCl.

Effects of repeated application of bradykinin

As shown in Fig. 5, when 10 $\mu g.ml^{-1}$ bradykinin was applied on the same receptive site in repetition with a 7 min interval, tachyphylaxis developed. The latency became longer and the discharge rate decreased in the subsequent trials. Repetition of bradykinin application up to five times was studied in twenty-two series using four

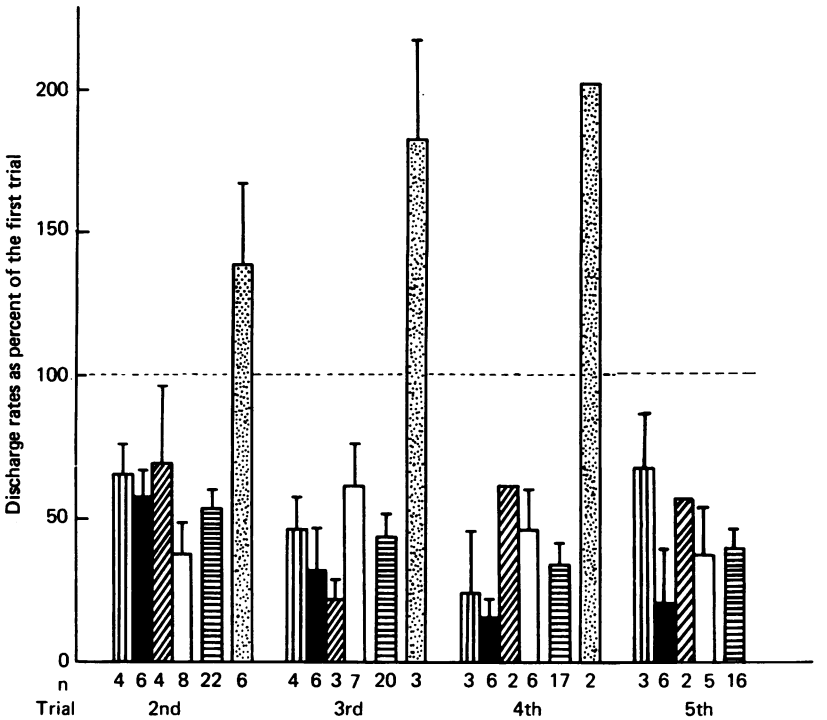

Fig. 6. Changes of responses to repeated applications of bradykinin of variable concentrations with short or long intervals between applications. Each column shows ratios (in percentage of the net increase of discharge) during a 60 s period of application of a trial to that of the first trial. Bars upon columns show S.E. of mean. Numbers of tested units are indicated under each column. The order of trial is shown at the bottom. Short interval: 6–14 min. Long interval: 30–60 min. Vertically hatched columns: 0·1 $\mu g.ml^{-1}$, short interval. Black columns; 0·3 $\mu g.ml^{-1}$, short interval. Obliquely hatched columns: 1 $\mu g.ml^{-1}$, short interval. White columns: 10 $\mu g.ml^{-1}$, short interval. Horizontally hatched columns: average of responses of 0·1, 0·3, 1 and 10 $\mu g.ml^{-1}$, short interval. Stippled columns: 1 $\mu g.ml^{-1}$, long interval. Note: marked reduction of response after short interval irrespective of the concentrations used. In contrast to this, varying degrees of enhancement of responses were seen after a long interval.

different concentrations, 0·1, 0·3, 1 and 10 $\mu g.ml^{-1}$. In these series the test was repeated with an interval less than 15 min. Discharge rates in the second and subsequent trials were much less in all except one among the total of seventy-five trials than those of the first trial of the series. These changes were observed irrespective of the magnitude of the first response. The response of the subsequent trials is expressed in percentage of that of the first trial, and average values of each concentration are shown as columns in Fig. 6. The tachyphylactic behaviour of bradykinin

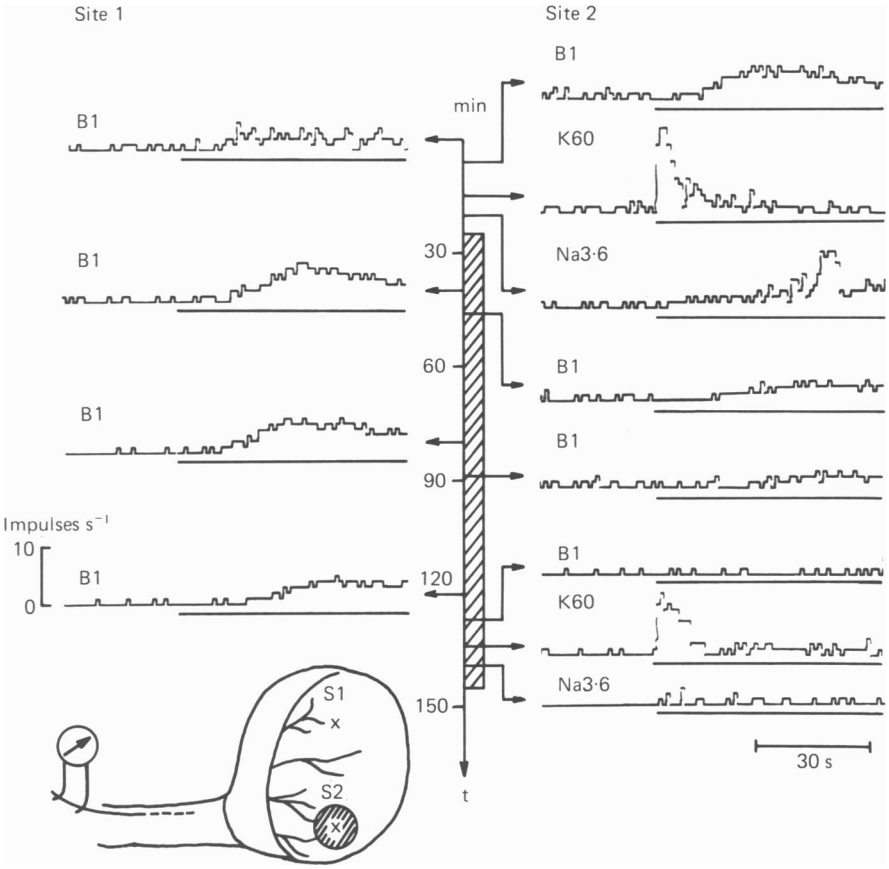

Fig. 7. An example of suppression of bradykinin response by indomethacin. Two receptive sites of a unit (depicted in the schematic drawing at the bottom left) were used, the one for testing bradykinin responses repeated every 40 min (S1), and the other for testing the effect of indomethacin on bradykinin response (S2). Responses of site 1 are shown on the left half, and site 2 on the right. The vertical bar in the centre is a time axis, and arrows attached to it show the time of application. Period of indomethacin (10 $\mu g.ml^{-1}$) application is shown as an obliquely hatched column. Chemicals used and concentration are shown at the upper left of each drawing. B1: bradykinin 1 $\mu g.ml^{-1}$, K 60: KCl 60 mmol.l^{-1}, Na 3·6; NaCl 3·6%. Conduction velocity: 7·7 m.s^{-1}. Receptive field: on the testis. Note: response to bradykinin 1 $\mu g.ml^{-1}$ in site 2 was apparently reduced 20 min after the start of indomethacin application, and complete reduction occurred 100 min after it, while response augumented in site 1. Compare the reduction of bradykinin response with weaker reduction of KCl response. This is the only unit in which indomethacin was tested on NaCl response. NaCl response was also markedly reduced after indomethacin.

responses did not differ substantially with the concentrations of the solution. The mean changes of the responses to solutions of all concentrations together reduced to $51{\cdot}8 \pm 7{\cdot}3$, $43{\cdot}0 \pm 8{\cdot}0$, $33{\cdot}8 \pm 7{\cdot}5$ and $38{\cdot}5 \pm 8{\cdot}3$% in the second to fifth trials respectively, indicating a remarkable reduction of response in the second trial.

The response to hypertonic NaCl at the same site did not change substantially after a marked reduction of the bradykinin response by repetitions as seen in Fig. 5.

A contrasting result was obtained when the response was tested with a longer interval (between 30 and 60 min) in six units using 1 $\mu g.ml^{-1}$ bradykinin. The mean response of the second to fourth trials shown by stippled columns in Fig. 6 was larger than the first response, though this increase of the discharge rate was not statistically significant due to the limited number of cases and large variations in degree of augmentation of each response. If the mean change shown by the stippled column in Fig. 6 is compared to that shown by the horizontally hatched column, they are significantly different in the second and third trial at a level of $P < 0{\cdot}01$ and $0{\cdot}001$ by the t test, respectively.

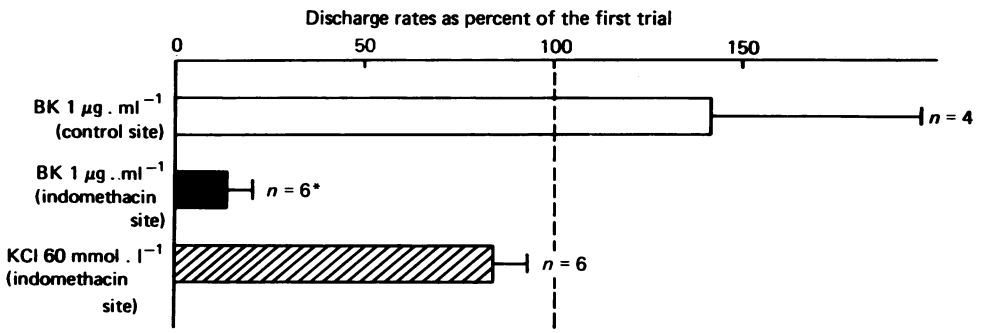

Fig. 8. Suppression of bradykinin response by indomethacin. The mean charge of discharges of the last trial, in percentage to discharges of the first trial, is depicted. White column: bradykinin 1 $\mu g.ml^{-1}$ on the control site; black column: bradykinin 1 $\mu g.ml^{-1}$ on the indomethacin site; hatched column: KCl 60 $mmol.l^{-1}$ on the indomethacin site. Bars attached to columns: S.E. of mean. n: number of tested sites. Note: marked reduction of bradykinin response (* $P < 0{\cdot}001$, by t test) on the indomethacin site, while an augmentation (though statistically not significant) of the response on the control site. KCl response shows a slight tendency for reduction, but it is insignificant.

Suppression of the response to bradykinin by indomethacin

Whether prostaglandins are involved in the action of bradykinin on the polymodal receptors was investigated by administration of indomethacin, which has been shown to be a potent inhibitor of the synthesis of prostaglandins (Vane, 1971).

Two receptive sites located well apart were chosen in the same unit, and the effect of indomethacin was tested at one of the two sites (S2) and the other was used as a control site (S1). After the response to 1 $\mu g.ml^{-1}$ bradykinin was tested at both sites, a thin layer of cotton soaked with 10 $\mu g.ml^{-1}$ of indomethacin was applied over an area that included S2. The pad was removed during testing with other agents. Subsequent testings of bradykinin were repeated with an interval between 30 and 60 min at both sites in order to avoid influences of tachyphylaxis. In addition, change of the response to 60 $mmol.l^{-1}$ KCl was tested at the indomethacin site. An example is shown in Fig. 7. At the control site, S1, bradykinin response tested every 40 min did not decrease but increased slightly; on the other hand at S2, bradykinin response tested with similar intervals decreased gradually, and 100 min after indomethacin it was almost abolished. Among seven units tested, indomethacin was ineffective in one unit, and in the other six units the response was reduced by 85 % on the aver-

age between 16 and 62 min after the application of indomethacin, while neither the response to 60 mmol.l^{-1} KCl at the same site nor that to 1 μg.ml^{-1} bradykinin at the control site changed significantly, as shown in Fig. 8.

In one unit after the response to 1 μg.ml^{-1} bradykinin was completely suppressed, 10 μg.ml^{-1} bradykinin, mechanical, and heat stimuli caused a substantial response.

As shown in Fig. 7, the response to 3·6 % NaCl was also greatly suppressed, though this was the only case tested.

DISCUSSION

Responses of the polymodal receptors of the scrotal contents to various chemicals are generally similar to those of the polymodal receptors in the skeletal muscle (Kumazawa & Mizumura, 1976, 1977*a*) in respect to the incidence of the response and discharge patterns to various chemicals. But the rapidly increasing and declining discharge pattern on application of KCl solution above 45 mmol.l^{-1} was different from that obtained in muscular polymodal receptor tested by close arterial injection of 60 mmol.l^{-1} KCl. A similar pattern, however, could be observed in the muscular units with much higher concentrations of KCl (cf. Fig. 2 of Mense, 1977). A different method of application of the solution might cause a weaker effect of KCl in the muscle experiment, although 4·5 % NaCl was equally effective in the muscle and scrotal contents.

Although solutions of NaCl, KCl and bradykinin caused responses in more than 90 % of polymodal receptors, the response patterns to these three chemicals differed substantially. This suggests that modes or sites of action are different with different chemicals. This possibility is supported by the finding that responses to KCl and NaCl were preserved after tachyphylaxis to bradykinin, and that the response to 60 mmol.l^{-1} KCl was not significantly changed by indomethacin at a time when the response to bradykinin was greatly reduced. As shown in Fig. 1 the discharge pattern to 30 mmol.l^{-1} KCl is quite different from that to concentrations above 45 mmol.l^{-1} KCl. This might suggest a different mode of action of the same chemicals depending on the concentration of the solution.

In visceral organs the response to bradykinin was considered to be elicited secondarily to contraction of smooth muscles of the intestine and the bladder (Taira, Nakayama & Hashimoto, 1968; Floyd, Hick, Koley & Morrison 1977). The bradykinin response of polymodal receptors in the present experiment was studied by application of bradykinin on the surface of the tunica vaginalis visceralis and thus involvement of mechanical events in that sense can be excluded, though the possibility of involvement of mechanical events in the blood vessels remains.

Repetition of bradykinin application with an interval less than 15 min caused a reduction of the response in the subsequent trials. This tachyphylactic behaviour to bradykinin was also found in the muscular polymodal receptors (Kumazawa & Mizumura, 1977*a*) and the cutaneous heat receptors (Beck & Handwerker, 1974). Neither the magnitude of the initial response nor the concentration of the solution caused a consistent difference in this phenomenon. In addition, the fact that the NaCl response was preserved after a remarkable reduction of the bradykinin response suggests that this phenomenon is not simply caused by the fatigue of the nerve endings. When

bradykinin was repeated with a longer interval, between 30 and 60 min, the response in the subsequent trials was enhanced in most cases, though statistically not significant. We have no definite explanation for these contrasting results at present. Lembeck, Popper & Juan (1976) found that release of prostaglandins by bradykinin increased several times when bradykinin was applied repeatedly with an interval of a few hours. Such a phenomenon as slow increase of prostaglandins might be involved in the enhanced response of polymodal receptors to repeated application of bradykinin with long intervals. Our observation of tachyphylaxis in the polymodal receptors conflicts with the observations of other investigators (Taira *et al.* 1968; Hiss & Mense, 1976). The cause of this discrepancy remains unsettled, but two contrasting phenomena found in the present experiment might explain these conflicting results.

It has been known that aspirin-like drugs suppress pseudo-affective reflexes (Hashimoto, Kumakura & Taira, 1964; Lim, Guzman, Rodgers, Goto, Braun, Dickerson and Engle, 1964; Ferreira, Moncada & Vane, 1973; Lembeck & Juan, 1974; Moncada, Ferreira & Vane, 1975) and also nociceptive unit discharges (Scott, 1968). Bradykinin activates release of prostaglandins in various tissues (McGiff, Terragno, Malik & Lonigro, 1972; Ferreira *et al.* 1973; Moncada *et al.* 1975; Lembeck *et al.* 1976) and prostaglandins enhance nociceptive reflexes (Juan & Lembeck, 1974). Indomethacin abolished prostaglandin release by bradykinin but did not completely antagonize pseudo-affective reflexes elicited especially by large doses of bradykinin (Ferreira *et al.* 1973; Moncada *et al.* 1975). In the present experiment, indomethacin substantially suppressed discharges of polymodal units induced by $1\ \mu g.ml^{-1}$ bradykinin but was much less effective against $10\ \mu g.ml^{-1}$ bradykinin. It is suggested that afferent discharges of polymodal receptors induced by bradykinin partly involve activation by prostaglandins released by the bradykinin applied.

Whether prostaglandins are involved in the response of the polymodal receptors to other kinds of stimuli is an interesting problem. King, Gallant, Myerson & Perl (1976) reported that indomethacin and carboxypeptidase B, singularly or both together, had little or no effect on heat sensitivity and heat sensitization of the cutaneous polymodal receptors. Juan & Lembeck (1974) reported that prostaglandin E_1 sensitized nociceptor response to KCl by a far less degree than those to bradykinin. Also in the present experiment, indomethacin did not suppress response induced by $60\ mmol.l^{-1}$ KCl significantly. But the effect of indomethacin might not be excluded in the response to KCl of the lower concentrations, where discharge patterns were quite different from those to higher doses of KCl. In regard to NaCl, though tested in only one unit, response to 3·6 % NaCl was substantially reduced by indomethacin. Release of prostaglandins by hypertonic NaCl solution has been reported in the kidney (Terashima, Anderson & Jubiz, 1976), but the time course of increase of prostaglandins seems to be too slow to be related to the rapid response of unit discharges observed in the polymodal receptors of the testis.

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