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MECHANISM OF BARORECEPTOR ADAPTATION IN DOGS: ATTENUATION OF ADAPTATION BY THE K^+ CHANNEL BLOCKER 4-AMINOPYRIDINE

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SUMMARY

1. Increased arterial pressure increases baroreceptor activity but activity declines (i.e. baroreceptors adapt) as the pressure is maintained at the higher level. The purpose of this study was to investigate the role of a 4-aminopyridine (4-AP)-sensitive K^+ current in causing baroreceptor adaptation.

2. Multi- and single fibre recordings of baroreceptor activity were obtained from the vascularly isolated carotid sinus in anaesthetized dogs during step increases in carotid sinus pressure sustained for periods up to 5 min.

3. Baroreceptor activity increased with the rise in pressure, declined markedly over the first minute, and continued to decline at a slower rate during the remainder of the 5 min period of elevated pressure. Exposure of the isolated carotid sinus to 4-AP (10^{-5} and 10^{-4} M) attenuated adaptation in a dose-dependent and reversible manner ($P < 0{\cdot}05$). 4-AP attenuated the gradual decline in single fibre activity and also prevented derecruitment or dropout of fibres that occurred over time. 4-AP did not alter peak nerve activity measured within the first 2 s of the pressure step.

4. Ouabain (5×10^{-7}–10^{-6} M), an inhibitor of Na^+, K^+-ATPase, increased baroreceptor activity but did not attenuate baroreceptor adaptation.

5. Neither 4-AP nor ouabain altered the distensibility of the carotid sinus as measured with sonomicrometer crystals suggesting that the agents act directly on the nerve endings.

6. The results suggest that activation of a 4-AP-sensitive K^+ current contributes significantly to baroreceptor adaptation with little or no contribution of Na^+, K^+-ATPase.

INTRODUCTION

Baroreceptor activity increases during a rise in arterial pressure but declines or adapts as the elevated pressure is maintained (Bronk & Stella, 1935; Landgren,

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1952; Munch, Andresen & Brown, 1983; Coleridge, Coleridge, Poore, Roberts & Schultz, 1984). A large portion of the adaptation occurs within the first minute after a rise in pressure, but nerve activity continues to decline at a slower rate for several minutes (Munch *et al.* 1983). This adaptation reduces the capacity of baroreceptors to buffer a sustained increase in pressure. The mechanism of baroreceptor adaptation is unclear and has been a subject of controversy (Coleridge *et al.* 1984; Heesch, Abboud & Thames, 1984; Coleridge, Coleridge, Poore, Roberts & Schultz, 1986; Munch & Brown, 1986; Krieger, 1987).

A recent study has provided evidence that a K^+ channel susceptible to blockade by 4-aminopyridine (4-AP) is present on baroreceptor terminals and is an important determinant of baroreceptor discharge (van Brederode, Seagard, Dean, Hopp & Kampine, 1990). Other studies have demonstrated that a 4-AP-sensitive K^+ current contributes to adaptation of various types of neurones during a sustained stimulus of constant intensity (Stansfeld, Marsh, Halliwell & Brown, 1986; Gean & Shinnick-Gallagher, 1989).

The purpose of this study was to examine whether a 4-AP-sensitive K^+ current contributes to adaptation of baroreceptors during an acute increase in arterial pressure. Baroreceptor responses to increases in carotid sinus pressure were obtained before and during exposure of baroreceptors to 4-AP. The results were contrasted with those obtained in the presence of ouabain, an inhibitor of Na^+, K^+-ATPase known to increase baroreceptor activity (Quest & Gillis, 1974; Saum, Brown & Tuley, 1976; Heesch *et al.* 1984).

METHODS

Mongrel dogs (18–22 kg) were anaesthetized with thiopental sodium (Pentothal, Abbott Laboratories, North Chicago, IL, USA; 30 mg/kg, I.V.) and α-chloralose (Sigma Chemical Co., St Louis, MO, USA; 80 mg/kg, I.V.). Supplemental doses of chloralose (5–10 mg/kg) were given at regular intervals to maintain a stable level of anaesthesia. The level of anaesthesia was monitored by examining the recording of arterial pressure. Increases in pressure or heart rate or irregular fluctuations in pressure indicated the need for additional anaesthetic. There was no cardiovascular response or pupillary dilatation during minor noxious stimuli such as pinching of the footpad. Dogs were mechanically ventilated with room air supplemented with oxygen, and blood gases were kept within normal limits by adjusting ventilation. Catheters were placed in a femoral artery and vein for measurement of systemic arterial pressure and providing anaesthesia, respectively. All procedures were in accordance with institutional guidelines.

Isolated carotid sinus preparation

The isolated carotid sinus preparation and method of recording baroreceptor activity have been described previously (Chapleau & Abboud, 1987; Chapleau, Hajduczok, Shasby & Abboud, 1988; Chapleau & Abboud, 1989) and are explained briefly. The carotid sinus was exposed and all arteries in the region of the sinus were ligated. Catheters were inserted in the common and external carotid arteries and the isolated sinus was flushed and filled with physiologic saline equilibrated with 95% O_2–5% CO_2 and warmed to approximately 37 °C. The vagosympathetic trunk and other nerves innervating the sinus region were cut.

The carotid sinus was connected to a pressurized fluid reservoir through the common carotid artery and pressure was measured through the external carotid artery (Statham transducer, model P23AA; Hato Rey, Puerto Rico). The level of pressure was controlled with a computer (IBM PC AT; Boca Raton, FL, USA) utilizing a series of solenoid valves located between the air pressure source and the fluid reservoir to ensure a reproducible rate of rise in pressure and maintenance of constant pressure over time.

Recording of baroreceptor activity

The carotid sinus nerve was sectioned near its junction with the glossopharyngeal nerve and desheathed. Multifibre recordings of baroreceptor activity were obtained from the whole nerve or a large nerve bundle after draping it across a bipolar platinum electrode connected to a Grass high impedance probe (model HIP 511E, Grass Instrument Co., Quincy, MA, USA). The nerve was encased in silicon gel (Wacker Sil-Gel) to prevent it from drying. Activity of single baroreceptor fibres was recorded after repeatedly splitting the nerve bundles until a fine filament was found that exhibited characteristics of single fibre discharge (Chapleau & Abboud, 1987; Chapleau *et al.* 1988; Chapleau & Abboud, 1989). Most of the fibres maintained relatively constant interspike intervals at constant pressure and based on their relatively high discharge frequency were probably of the medullated type. The entire region of the carotid sinus was covered with paraffin oil. Nerve traffic was amplified with a Grass bandpass amplifier (model P511; low pass cut-off 30 Hz, high pass cut-off 3000 Hz), viewed on an oscilloscope (Tektronix, model 5113, Beaverton, OR, USA) and heard through a loudspeaker. The gas tensions and pH of the physiological saline and arterial blood minimized chemoreceptor activity. The value of pH was between 7·35 and 7·45, the P_{CO_2} between 25 and 40 mmHg, and the P_{O_2} between 200 and 300 mmHg. Decamethonium bromide (Syncurine, ICN-K & K Laboratories, Cleveland, OH, USA; 0·3 mg/kg, I.V.) was administered to prevent skeletal muscle contraction when nerve activity was recorded. The dose of chloralose administered was sufficient to provide adequate anaesthesia and analgesia as demonstrated in dogs in the absence of decamethonium.

Baroreceptor activity was quantified by counting the number of action potentials that exceeded a selected voltage with a nerve traffic analyser (model 706C, Bioengineering, University of Iowa, Iowa City, IA, USA). In single fibre experiments, spike counts were confirmed by counting spikes manually from the neurogram played back at fast paper speed. The output from the spike counter and measurements of carotid sinus and systemic arterial pressure were registered on a Beckman dynograph recorder (model R411, Schiller Park, IL, USA). These variables and the raw neurogram of carotid sinus nerve activity were recorded, digitized, and stored on VHS tape with a digital processor and VHS recorder (Vetter Co., model 4000, Rebersburg, PA, USA).

Recording of carotid sinus diameter

The diameter of the carotid sinus was measured with sonomicrometer crystals (Pagani, Baig, Sherman, Manders, Quinn, Patrick, Franklin & Vatner, 1978; Chapleau *et al.* 1988; Chapleau & Abboud, 1989). Two 5 MHz piezoelectric crystals, mounted on opposite ends of a low resistance stainless-steel clip were positioned across the carotid sinus. The clip was held in place by suturing the Dacron mesh glued to one side of the clip to the adventitia of the sinus. The measurement of diameter was derived from the transit time of the acoustic signal between the two crystals and was displayed on the Beckman recorder.

Protocols

Multifibre baroreceptor activity. Baroreceptor activity was recorded from multifibre preparations in twelve dogs during step increases in non-pulsatile carotid sinus pressure that were sustained for periods of 5 min. Pressure was held at a low control level of 40 mmHg for 5–10 min before each step increase in pressure. In each experiment, pressure was raised to several levels. The data reported represent responses of multifibre activity to a single level of elevated pressure (93 ± 5 mmHg) where adaptation was marked. The maximum rate of rise in pressure (dP/dt) was approximately 100–150 mmHg/s and was constant within each experiment. A reproducible rise in pressure and maintenance of constant pressure over time were achieved with a computer and selenoid valves. Carotid sinus pressure was sampled at an average rate of 300 Hz and digitized, providing feedback to the computer for control of pressure.

Baroreceptor responses to identical increases in pressure were measured both before ($n = 6$) and during ($n = 5$) exposure of the carotid sinus to 4-AP (Sigma; 10^{-5} and 10^{-4} M) and after removal of 4-AP from the sinus ($n = 5$). Experiments were performed in separate animals to assess baroreceptor responses before and during exposure of the carotid sinus to ouabain (Sigma; 5×10^{-7}–10^{-6} M, $n = 6$). In two of these latter experiments, responses were obtained again after exposure to 4-AP (10^{-4} M). The carotid sinuses were exposed to the drugs intraluminally for an average of 26 ± 4 min before the pressure steps were applied.

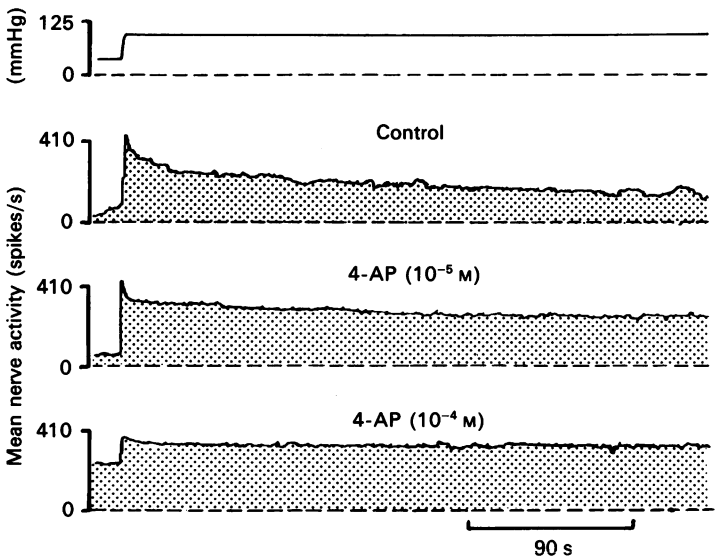

Fig. 1. Original recordings of mean multifibre baroreceptor activity during step increases in carotid sinus pressure (CSP) from 40 to 100 mmHg. The recording of carotid sinus pressure is shown only once for clarity. 4-Aminopyridine (4-AP) caused a dose-related attenuation of baroreceptor adaptation without influencing the peak nerve activity measured within the first 2 s of the rise in pressure. As a result, 4-AP increased the level of activity measured after 5 min of elevated pressure.

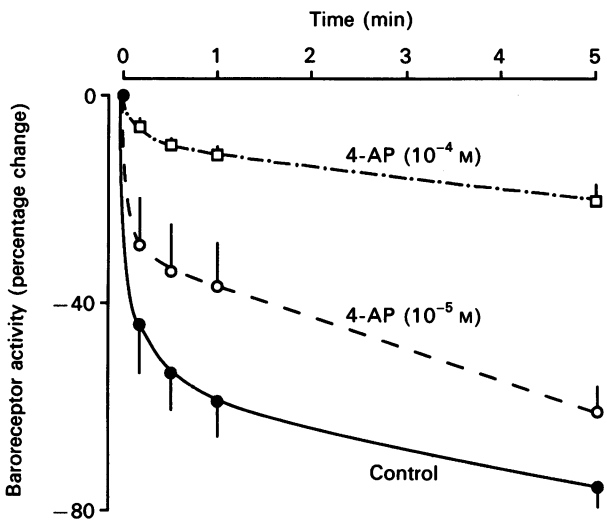

Fig. 2. Progressive decreases in multifibre baroreceptor activity that occurred over time after a step increase in carotid sinus pressure from 40 to 92±5 mmHg in absence ($n = 6$) and presence ($n = 5$) of 4-aminopyridine (4-AP). The rapid phase of adaptation of activity (0–1 min) was significantly attenuated by 4-AP in a dose-dependent manner. The slow phase of adaptation (1–5 min) was significant under control conditions and was eliminated, i.e. activity did not change significantly between 1 and 5 min, in the presence of the higher concentration of 4-AP (10^{-4} M) (two-factor ANOVA and contrast testing, $P < 0.05$).

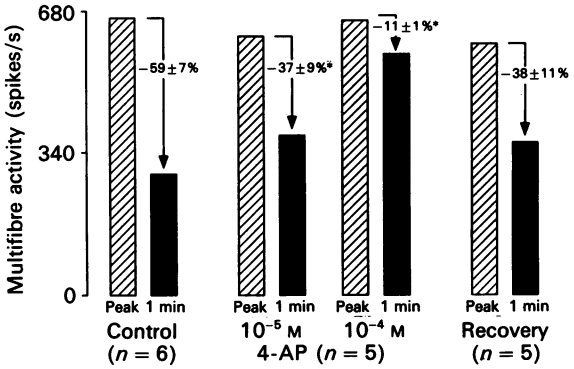

Fig. 3. Influence of 4-AP on the rapid phase of adaptation of multifibre baroreceptor activity. Peak nerve activity recorded within 2 s of the pressure step (40 to 92±5 mmHg) and the adapted level of activity after 1 min of elevated pressure are shown during control ($n = 6$), 4-AP (10^{-5}, 10^{-4} M, $n = 5$), and recovery ($n = 5$). The percentage decreases in activity that occurred over the 1 min periods are shown above the bars. * indicates significant attenuation of adaptation by 4-AP (Student's paired t test with Bonferroni correction for multiple comparison, $P < 0.05$).

TABLE 1. Influence of 4-aminopyridine (4-AP) on baroreceptor activity

	Baseline activity at 40 mmHg (spikes/s)	Peak discharge frequency at 92±5 mmHg (spikes/s)
Control ($n = 6$)	66±19	663±96
4-AP		
10^{-5} M ($n = 5$)	113±35	620±100
10^{-4} M ($n = 5$)	295±45*	657±97
Recovery ($n = 5$)	109±30	602±84

Data represent means±S.E.M.

Peak discharge frequency was measured within 2 s of the rise in pressure.

* Significant difference compared with control, $P < 0.05$.

Measurements were made of multifibre activity at 40 mmHg and peak discharge frequency in response to pressure step.

Single fibre baroreceptor activity. Nerve activity was recorded from ten single baroreceptor fibres in nine dogs. A similar protocol as described above for multifibre recordings was performed. Non-pulsatile carotid sinus pressure was raised from a low level (40–70 mmHg) to various levels of elevated pressure ranging from 80 to 175 mmHg. The pressure steps were maintained for 5 min unless the fibre ceased to discharge before 5 min had passed. The influence of 4-AP (10^{-5}, 10^{-4} M) on baroreceptor adaptation was examined in six single fibres in five dogs. The influence of ouabain (10^{-6} M) was examined in separate animals (four fibres in four dogs).

Pressure–diameter relation of carotid sinus. In seven additional experiments, the influences of 4-AP (10^{-4} M, $n = 4$) and ouabain (10^{-6} M, $n = 3$) on the pressure–diameter relation of the carotid sinus were determined. Pressure was raised from 40 to 180 mmHg in steps of 20 mmHg. Each level of pressure was maintained for approximately 60 s and values of diameter were obtained 20–40 s after each step in pressure.

Data analysis

Adaptation was quantitated as the magnitude of decline in nerve activity that occurred over time during the period of elevated pressure. The level of activity measured 10 s, 30 s, 1 min, and 5 min after the initial rise in pressure was subtracted from the peak activity measured within 2 s of the rise in pressure and the percentage decline in activity was calculated. When a single fibre ceased to discharge during the 5 min of elevated pressure, the duration of discharge was measured.

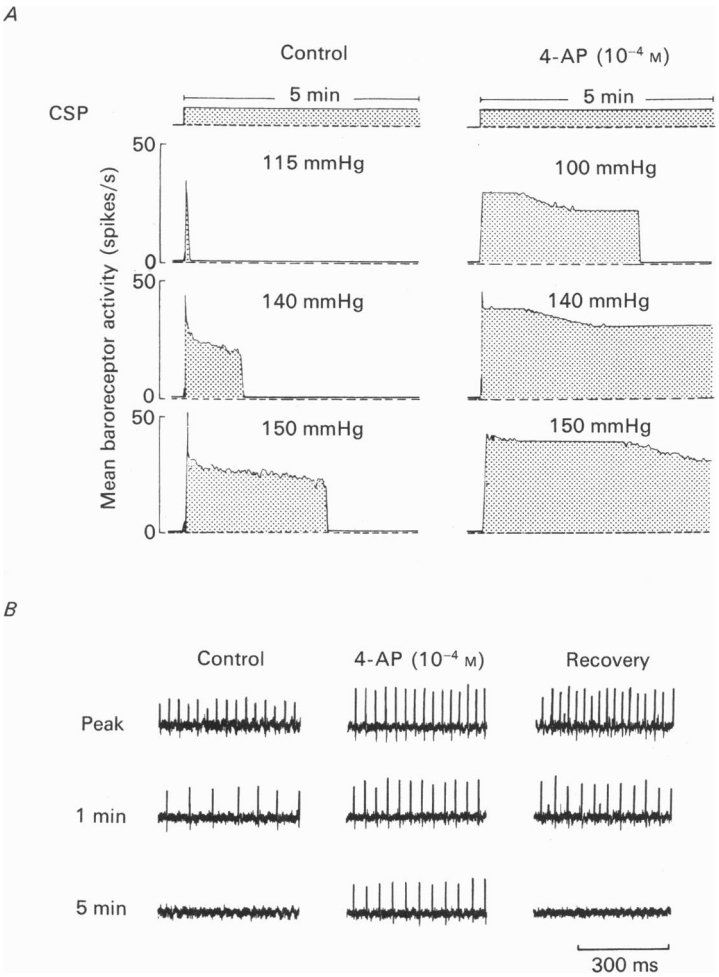

Fig. 4. Influence of 4-AP on adaptation of activity of a single baroreceptor fibre during sustained increases in carotid sinus pressure. *A*, recordings of mean discharge frequency in response to step increases in pressure to several levels during control (left) and 4-AP (right). 4-AP attenuated the decline in nerve activity that occurred over time and prolonged the duration of continuous discharge. *B*, neurograms recorded during the peak response to increasing pressure to 140 mmHg, after 1 min and 5 min of pressure elevation are shown for control, 4-AP, and recovery (same fibre as *A*).

The influences of 4-AP and ouabain on the magnitude of baroreceptor adaptation over time and on the pressure–diameter relation were analysed by two methods, two-way analysis of variance (ANOVA) and contrast testing (Keppel, 1982). The influence of these drugs on baseline and peak

nerve activity, the magnitude of adaptation during the first minute of the pressure step, and the slope of the pressure–diameter relation were analysed by Student's paired t test (Snedecor & Cochran, 1980). The Bonferroni test was used to correct for multiple comparisons when more than one comparison was made (Wallenstein, Zucker & Fleiss, 1980). The data represent means $\pm$ standard error of the mean (S.E.M.). Differences were considered significant when $P < 0{\cdot}05$.

RESULTS

Effect of 4-AP on baroreceptor adaptation

Multifibre baroreceptor activity increased as carotid sinus pressure was raised from 40 to 92 ± 5 mmHg and then declined back towards control as the elevated pressure was maintained constant ($n = 6$, Figs 1 and 2). Most of the adaptation

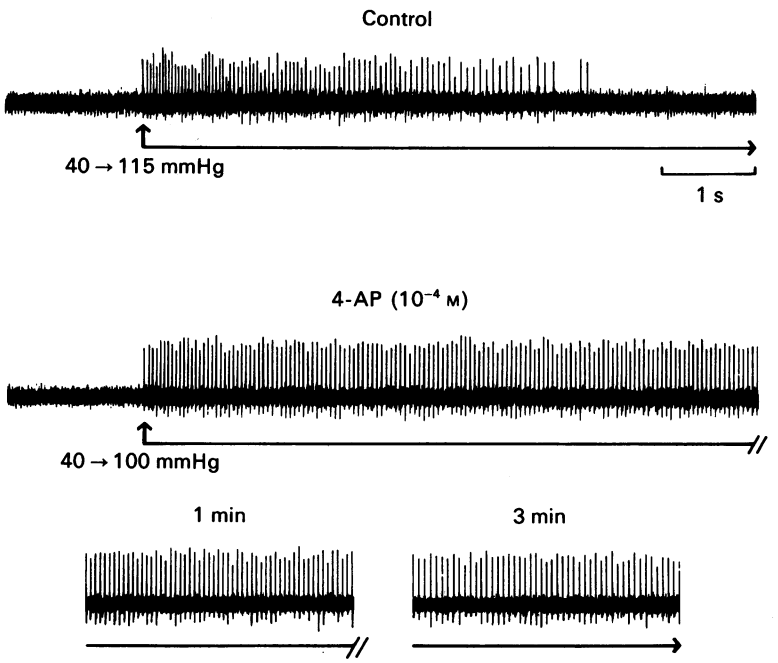

Fig. 5. Neurogram recorded from a single baroreceptor fibre during pressure step from 40 to 115 mmHg in control condition and from 40 to 100 mmHg in presence of 4-aminopyridine (4-AP, 10^{-4} M). During control, the baroreceptor was activated by the rise in pressure, adapted, and ceased to discharge as the elevated pressure was maintained. In contrast, during exposure to 4-AP nerve activity was sustained with minimal adaptation. The arrows indicate the periods of elevated pressure.

occurred rapidly during the first minute with a slower rate of adaptation over the rest of the 5 min period of elevated pressure (Figs 1 and 2). Exposure of the carotid sinus to 4-AP increased baseline nerve activity at 40 mmHg but did not alter peak discharge frequency significantly in response to a step increase in pressure (Fig. 1, Table 1). Application of 4-AP significantly attenuated baroreceptor adaptation in a dose-dependent manner (Figs 1–3). Adaptation was partially restored after removal of 4-AP from the isolated carotid sinus (Fig. 3).

The activity of single baroreceptor fibres increased abruptly with the rise in pressure, decreased rapidly at first, then gradually, and often ceased abruptly despite the maintained increase in pressure (Figs 4 and 5). Both the level of baroreceptor activity and the duration of discharge were positively related to the level of carotid sinus pressure (Fig. 4, Table 2). 4-AP did not increase the peak activity during the rise in pressure but significantly attenuated the decline in activity and prevented or delayed the abrupt cessation of activity as the elevated pressure was maintained (Figs 4–6, Table 2).

TABLE 2. Influence of level of pressure and 4-aminopyridine (4-AP) on duration of continuous discharge of individual baroreceptor fibres

	During control		In presence of 4-AP	
	Pressure step (mmHg)	Duration (s)	Pressure step (mmHg)	Duration (s)
Fibre 1	40–80	24	40–80	300+
	40–88	65	40–90	300+
	40–92	142	40–100	300+
	40–120	300+	40–120	300+
Fibre 2	40–80	21	40–80	151
	40–90	176	40–90	300+
Fibre 3	40–80	118	40–80	300+
	40–90	300+	—	—
Fibre 4	40–117	5	40–97	207
	40–137	75	40–139	300+
	40–148	185	40–148	300+
Fibre 5	70–134	4	70–134	120
	70–155	19	70–165	208
	70–175	70	70–170	258
Fibre 6	40–88	300+	40–80	300+

Concentration of 4-AP was 10^{-4} M for fibres 1, 4, 5 and 6, and 10^{-5} M for fibres 2 and 3. (300+ indicates that the fibre continued to discharge for the entire 5 min (300 s) period, at which time pressure was lowered.)

The duration of discharge of single units in response to a sustained increase in pressure from 46 ± 6 to 112 ± 19 mmHg averaged $1{\cdot}5 \pm 0{\cdot}4$ min ($n = 5$). In the presence of 4-AP, these same fibres continued to discharge for the entire 5 min period of elevated pressure (112 ± 18 mmHg) in four of five experiments and for over 4 min in the remaining experiment.

In three of the six single fibres, recovery from 4-AP was tested. Removal of 4-AP from the isolated carotid sinus restored adaptation of single fibre activity. Within the first minute after the step increase in pressure, nerve activity declined by $37 \pm 14\%$ in control, $13 \pm 4\%$ during 4-AP, and $28 \pm 9\%$ during recovery (also see Fig. 4*B*).

Effect of ouabain on baroreceptor adaptation

Ouabain (5×10^{-7}–10^{-6} M, $n = 10$) significantly increased the peak discharge frequency of baroreceptors measured within the first 2 s of a pressure step in both single ($n = 4$) and multifibre ($n = 6$) preparations. After raising pressure from 40 to

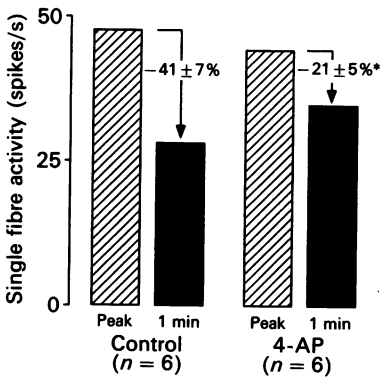

Fig. 6. Influence of 4-AP on adaptation of single baroreceptor fibre activity. Peak nerve activity and the activity after 1 min of elevated pressure (110±15 and 108±15 mmHg for control and 4-AP, respectively) are shown ($n = 6$). The percentage decreases in activity that occurred over the 1 min periods are shown above the bars. At these levels of elevated pressure each of the six fibres remained active for the entire 1 min period. * indicates significant attenuation of adaptation by 4-AP ($P < 0.05$).

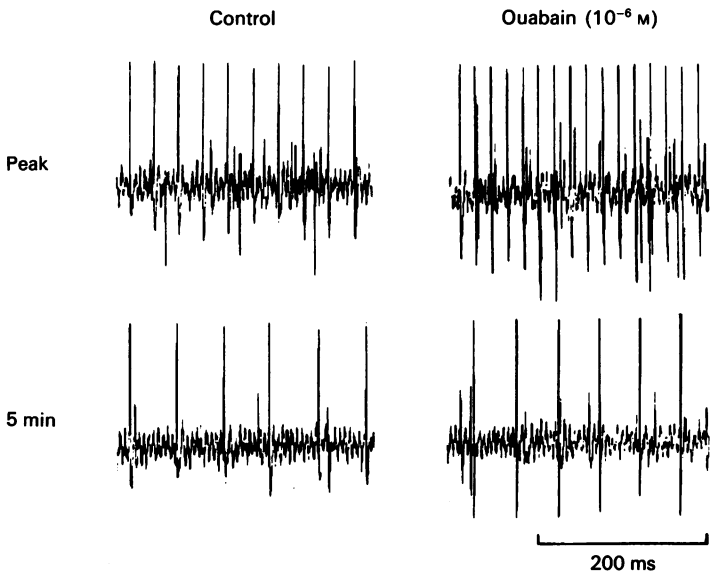

Fig. 7. Neurogram of single baroreceptor fibre during peak response to increase in pressure from 40 to 120 mmHg and after 5 min of elevated pressure before (left) and during exposure of carotid sinus to ouabain (right). Ouabain increased peak discharge frequency but did not influence adaptation.

108±9 mmHg, peak discharge frequency averaged 241±75 spikes/s before and 276±80 spikes/s during exposure to ouabain, an increase of 18±4%. Ouabain did not influence baroreceptor adaptation significantly (Figs 7–9).

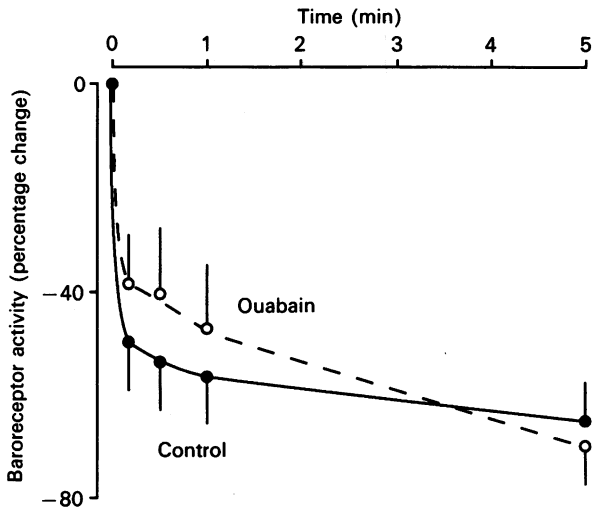

Fig. 8. Progressive decreases in multifibre baroreceptor activity that occurred over time after a step increase in pressure from 40 to 93±8 mmHg in presence and absence of ouabain ($n = 6$). Baroreceptor adaptation was not influenced significantly by ouabain (two-factor ANOVA).

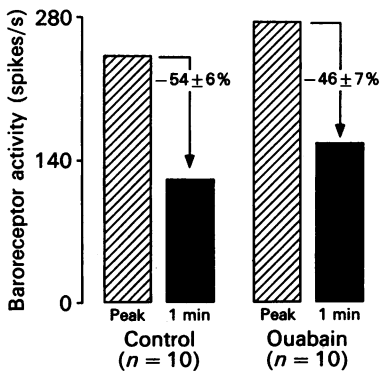

Fig. 9. Influence of ouabain on the adaptation of baroreceptors that occurred within the first minute of the pressure step ($n = 10$). Peak nerve activity and the activity after 1 min of elevated pressure (108±9 mmHg) are shown during control and ouabain (5×10^{-7}–10^{-6} M). The percentage decreases in activity that occurred over the 1 min periods are shown above the bars. Adaptation was not influenced significantly by ouabain.

In two experiments, 4-AP (10^{-4} M) given after ouabain, reduced the adaptation of multifibre baroreceptor activity. The decrease in nerve activity over the 5 min period of elevated pressure averaged 77% in control, 70% during ouabain, and 48% during 4-AP.

4-AP and ouabain and pressure–diameter relation

Neither 4-AP ($n = 4$) nor ouabain ($n = 3$) significantly influenced the diameter of the carotid sinus at any particular level of pressure nor did they alter the slope of the pressure–diameter relation (Fig. 10).

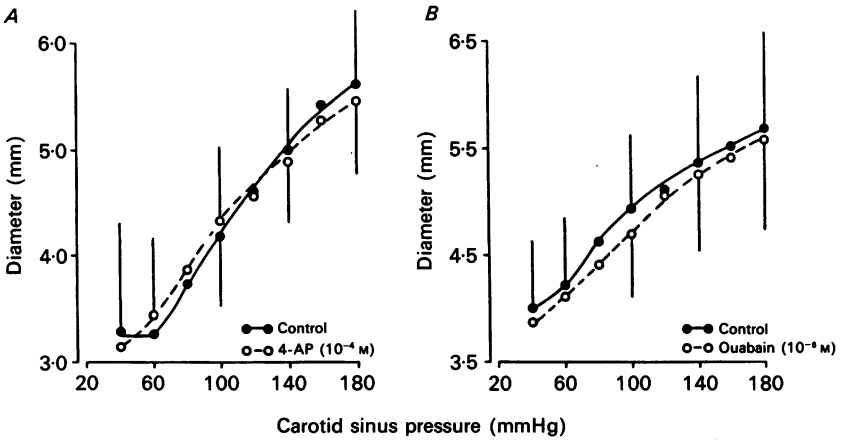

Fig. 10. Effect of 4-AP (*A*, $n = 4$) and ouabain (*B*, $n = 3$) on the pressure–diameter relation of the isolated carotid sinus. Neither agent significantly influenced the pressure–diameter curve.

DISCUSSION

The major new finding in this study is that 4-AP attenuated the adaptation of baroreceptors during a sustained increase in carotid sinus pressure. Since 4-AP did not alter the carotid sinus pressure–diameter relation, its influence on adaptation was probably mediated through a direct effect on baroreceptors. Ouabain which inhibits Na^+,K^+-ATPase increased baroreceptor activity but did not attenuate adaptation. The results suggest that a 4-AP-sensitive K^+ current contributes significantly to baroreceptor adaptation during acute hypertension.

Activation of K^+ channels has often been invoked as a mechanism contributing to adaptation in other cells including neurones (Husmark & Ottoson, 1971; Ottoson & Swerup, 1982; Stansfeld *et al.* 1986; Gean & Shinnick-Gallagher, 1989). Many types of K^+ channels exist in cells that vary in their voltage dependence, pharmacology, and kinetics, and subserve different functions (Rogawski, 1985; Rudy, 1988). 4-AP is classically used to block 'transient' K^+ channels, referred to as 'A-current', in a relatively selective manner. These channels are activated by depolarization originating from a relatively hyperpolarized membrane potential and are rapidly inactivated with maintained or additional depolarization (Rogawski, 1985; Rudy, 1988). The channels play an important role in controlling the rate of depolarization within the interspike interval and in determining neuronal excitability and discharge frequency. Tetraethylammonium, which is a potent blocker of delayed rectifier K^+ channels (Rogawski, 1985; Rudy, 1988), has minimal effects on 'A-current'. In experiments using a different protocol we observed that tetraethylammonium

(Sigma; 10^{-5}, 10^{-4} M) placed in the isolated sinus did not influence baroreceptor adaptation. In most cells 4-AP has little or no effect on delayed rectifier channels at concentrations within the range that we have used to block the 'A-current' (Rogawski, 1985; Rudy, 1988).

In a recent study (van Brederode *et al.* 1990), 4-AP decreased the pressure threshold required to trigger activity in a subpopulation of single baroreceptor units classified as Type I. The authors concluded that activation of a 4-AP-sensitive K^+ channel suppresses activation of Type I baroreceptors at pressures below threshold (van Brederode *et al.* 1990). Consistent with our results, 4-AP did not significantly alter the carotid sinus pressure–volume relation suggesting that 4-AP acts directly on the afferent neurones rather than indirectly through changes in vascular tone.

In our experiments, 4-AP attenuated baroreceptor adaptation and prevented or delayed the derecruitment of single units that occurred over seconds to minutes after a step increase in carotid sinus pressure (Figs 1–6, Table 2). Since voltage clamp studies have demonstrated that K^+ channels responsible for 'A-current' usually inactivate within 100 ms of a depolarizing voltage step (Rogawski, 1985; Rudy, 1988), their involvement in the phenomenon of adaptation of baroreceptors may be questioned. We offer two possible explanations in support of our interpretation. First, the kinetics of inactivation of 4-AP-sensitive K^+ channels vary among different types of cells. K^+ channels have been identified that inactivate relatively slowly over a period of seconds or do not inactivate completely, yet are blocked by micromolar concentrations of 4-AP similar to the concentrations we have used (Stansfeld *et al.* 1986; Rudy, 1988). A subpopulation of visceral afferent neurones from nodose ganglia (site of baroreceptor neurone cell bodies) possess a slowly inactivating K^+ current that is inhibited by 4-AP (Stansfeld *et al.* 1986). Low concentrations of 4-AP (1–30 μM) inhibit spike adaptation during injection of a constant depolarizing current in these cells (Stansfeld *et al.* 1986).

Second, the results obtained in cells under voltage clamp may not reflect the kinetics of the 4-AP-sensitive K^+ current during action potential generation *in vivo*. Under voltage clamp conditions, the maintained membrane depolarization may lead to K^+ channel inactivation but *in vivo*, the repetitive discharge of action potentials associated with hyperpolarization after each spike may enable periodic reactivation of K^+ channels for extended periods during elevated pressure.

As in previous studies (Bronk & Stella, 1935; Landgren, 1952; Munch *et al.* 1983; Coleridge *et al.* 1984), we found that baroreceptor activity declines or adapts most rapidly within the first few seconds after a rise in arterial pressure. Nerve activity is relatively constant within 1 min but continues to decline slowly over the next 5–15 min (present results; Munch *et al.* 1983). Most of the previous studies that have investigated baroreceptor adaptation have measured responses within the first minute of elevated pressure. Therefore, we have focused our analysis primarily on this time period (Figs 3, 6, and 9).

The slower decline in nerve activity that we observed to occur between 1 and 5 min after the pressure step coincides with the duration of hypertension often used to cause acute resetting of the baroreceptor pressure–activity curve (Munch *et al.* 1983; Coleridge *et al.* 1984; Heesch *et al.* 1984). We did not determine pressure–activity curves and therefore did not investigate the influence of 4-AP on acute baroreceptor

resetting. Since 'adaptation' can generally be defined as a decline in response during a maintained stimulus of constant intensity, we believe it is appropriate to refer to the decline in baroreceptor activity at constant pressure over this longer period as 'adaptation'. The phenomenon of 'adaptation', particularly over the longer time period, and 'acute resetting' may therefore share a common mechanism and/or more than one mechanism may be operative simultaneously.

Activation of an electrogenic Na^+ pump contributes to neural adaptation during stretch (Nakajima & Onodera, 1969; Sokolove & Cooke, 1971) and to postexcitatory depression of activity after a period of increased activity (Nakajima & Takahashi, 1966) in crayfish stretch receptors. Previous studies of baroreceptor function have shown that inhibition of the Na^+ pump (e.g. with ouabain) increases baroreceptor activity and prevents postexcitatory depression of nerve activity and resetting of the pressure threshold after periods of increased pressure (Quest & Gillis, 1974; Saum *et al.* 1976; Heesch *et al.* 1984). Although activation of the Na^+ pump appears to be important in reducing baroreceptor responsiveness *after* periods of hypertension, the finding that inhibition of the Na^+ pump with ouabain or low K^+ solutions does not attenuate adaptation (Saum *et al.* 1976 and present results) suggests that pump activation does not contribute to baroreceptor adaptation *during* the period of elevated pressure even between 1 and 5 min after the pressure step (Fig. 8). As suggested by Saum *et al.* (1976), it is possible that the high membrane conductance present during elevated pressure minimizes the relative contribution of the Na^+ pump to membrane potential. In contrast, once pressure is lowered the same pump activity may trigger significant membrane hyperpolarization (Saum *et al.* 1976).

Our results suggest that a 4-AP-sensitive K^+ current contributes to baroreceptor adaptation, including the slow decline in activity that occurs between 1 and 5 min after the pressure step. The decline in activity over this period was significant during control but was no longer significant ($P > 0{\cdot}05$) in the presence of 4-AP (Fig. 2). A preliminary report suggesting that 4-AP does not attenuate acute resetting of the baroreceptor pressure–activity curve following a 20 min period of elevated carotid sinus pressure (Drummond, Van Wynsberghe, Hopp & Seagard, 1992) is consistent with resetting being mediated by other mechanisms such as Na^+ pump activation (Heesch *et al.* 1984). Thus, different mechanisms appear to mediate baroreceptor adaptation and acute resetting of the pressure threshold.

It should be noted that some adaptation of baroreceptors was still evident in the presence of 4-AP (Figs 1–6) which may reflect incomplete block of channel activity or the contribution of a different mechanism such as viscoelastic creep (Coleridge *et al.* 1984; Krieger, 1987). During a sustained rise in pressure, relaxation of elements of the vascular wall (creep) coupled in series with the nerve endings may progressively reduce tension on the nerve endings (Coleridge *et al.* 1984; Krieger, 1987). Changes in ionic concentration gradients, for example intracellular or extracellular K^+, may also contribute to adaptation.

The method we used to calculate adaptation requires some comment. Adaptation was calculated as the percentage decrease in nerve activity that occurred over time during the period of elevated pressure ('peak activity' − 'adapted activity'/'peak activity'). Thus, the level of baseline activity prior to the pressure step was not taken into account in the calculation. Previous studies examining baroreceptor adaptation

also focused on the decline in activity during the period of elevated pressure without consideration of baseline values prior to the pressure step (Bronk & Stella, 1935; Landgren, 1952; Quest & Gillis, 1974; Saum *et al.* 1976; Munch *et al.* 1983; Coleridge *et al.* 1984). The rate of rise (dP/dt) and absolute level of elevated pressure have been considered the primary factors determining peak and steady-state discharge frequency of baroreceptors (Landgren, 1952). Both these factors were maintained constant in our experiments. Nevertheless, it is possible that increased baseline activity, as occurred with 4-AP in the multifibre preparations, might limit the decline in nerve activity over time during elevated pressure. Therefore, we also calculated adaptation relative to the baseline level of activity ('peak activity' − 'adapted activity'/'peak activity − baseline activity') which takes into account changes in baseline discharge frequency. 4-AP also significantly attenuated adaptation calculated in this way. The decline in multifibre activity over the first minute of elevated pressure averaged $66 \pm 8\%$ in control and 45 ± 9 and $25 \pm 3\%$ during exposure to 10^{-5} and 10^{-4} M 4-AP, respectively ($n = 6$, $P < 0{\cdot}05$). In addition, 4-AP attenuated adaptation in single fibres (Figs 4–6) which were not active prior to the pressure step. Only one of six fibres demonstrated increased baseline activity during 4-AP; the other five fibres remained inactive most probably because the baseline level of pressure was much lower than the pressure threshold for these fibres.

The increased multifibre activity at 40 mmHg in the presence of 4-AP is consistent with results of van Brederode *et al.* (1990), who demonstrated a decrease in pressure threshold by 4-AP. Decreased pressure threshold of single fibres would result in a greater number of fibres active at 40 mmHg and increased baseline multifibre activity.

The results of our study may have provocative implications concerning the role of baroreceptors in buffering an initial rapid increase in arterial pressure *vs.* a sustained increase in pressure. As arterial pressure increases, the increase in baroreceptor activity triggers reflex responses that buffer the rise in pressure. If the increase in pressure is maintained, adaptation of activity may occur. The decline in baroreceptor activity would prevent sustained buffering of the rise in pressure. If activation of a 4-AP-sensitive K^+ current impairs the ability of baroreceptors to buffer a sustained increase in pressure, the inhibition of this current may, by preventing adaptation, enhance the ability to buffer a sustained rise in pressure. 4-AP-sensitive K^+ currents are subject to physiological modulation (Rogawski, 1985). Therefore, it is reasonable to propose that inhibition of K^+ current under certain conditions may enhance the ability to buffer a sustained increase in arterial pressure.

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