Effect of norepinephrine and vasopressin on carotid sinus baroreceptor activity in the anesthetized rabbit[1]

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Summary. Norepinephrine (NE) 10^{-6} M or vasopressin (VP) 12.5 μU/ml were injected into the isolated carotid sinus of anesthetized rabbits. The sinus was exposed either to the arterial pressure or to a pressure controlled reservoir. Multifiber and single fiber recordings were made. Both NE and VP increased baroreceptor activity at all sinus pressures but decreased activity in a few fibers. The results are consistent with the drugs having their effects on smooth muscle fibers in the adventitia.

The region of the carotid sinus has an efferent adrenergic innervation which it is believed may modify the baroreceptor reflex through release of norepinephrine from the nerve terminals[2]. Modification of the baroreceptor reflex may occur secondarily to contraction of smooth muscle elements in the adventitia, to a decrease in sinus diameter[3] or to direct sensitization of the baroreceptor endings. Direct application of norepinephrine (NE) to the sinus was reported to increase baroreceptor discharge frequency, with the increase being mainly in spikes recorded with low amplitude and in late diastole[4]. Administration of vasopressin (VP) was reported to increase the frequency of both large and small amplitude spikes[4]. Later studies supported the finding that NE affected mainly small amplitude spikes[5,6]. More recently investigators have disagreed with this view. Tomomatsu and Nishi[7] reported that NE perfused through the sinus at 10^{-9} M increased the firing of all fibers tested. Interestingly they found that NE 10^{-6} M did not change baroreceptor discharge. Bergel et al.[8] reported that NE caused baroreceptors to decrease their firing rate at low sinus pressures but increase their firing rate at high sinus pressures. We have been unable to find recent studies of the effects of VP on baroreceptor discharge or any comparison of the effects of VP and NE. Since both agents contract smooth muscle, differences in their effects on the baroreceptors may provide evidence of direct activation of the receptors.

We have examined in anesthetized rabbits the effect of injection into the isolated carotid sinus of known concentrations of NE and VP at controlled sinus pressures. We also made injections into the closed sinus exposed to normal pulsatile pressures since Bagshaw and Peterson[9] had suggested that NE might alter the dynamic response of the receptors without affecting the static response. Recordings were taken from multifiber preparations and from preparations with a single functional fiber of the carotid sinus nerve.

Materials and methods. Experiments were carried out in albino rabbits of 1.4–3.0 kg. The results presented were obtained from 6 rabbits; recordings from 1–4 different fibers were obtained from each rabbit. The rabbits were anesthetized with urethane (1.2 mg/kg) and barbitone (30 mg/kg) i.p. The trachea was cannulated and the animal ventilated (Harvard, model 614) at 22 to 26 strokes/min and volume of 75 ml. Intratracheal pressure was limited by allowing a leak through a Y-piece attached to the tracheal cannula. Arterial samples were taken for gas analysis and the respiration pump adjusted to maintain P_{CO_2} between 3.9–4.6 kPa. Plasma pH was adjusted to 7.37 by administration of sodium bicarbonate (1 M). Body temperature was kept at 37 °C by means of a heated table. Arterial pressure was recorded from the femoral artery.

The carotid sinus on one side was vascularly isolated but the carotid artery was left intact. The lingual artery was cannulated (Intramedic, PE-50) and used for recording of carotid sinus pressure. The external carotid artery was also cannulated (Intramedic, PE-50) and connected to a Y-piece one arm being for injection of drugs the other leading to a pressure bottle containing Ringer's solution at 37 °C. To isolate the sinus completely a clip was placed on the common carotid artery but this was removed except during control of pressure, to allow perfusion of the sinus with blood.

The carotid sinus nerve was located at its junction with the glossopharyngeal nerve, cut and dissected free of connective tissue. It was laid on a dissecting platform, covered with mineral oil and the sheath removed. Recordings were made from the whole nerve, or the nerve was split until single functional fibers were obtained. Activity was recorded with bipolar platinum electrodes, amplified (Grass Inst. Co., P15, 100–3000 Hz) and displayed on an oscilloscope (Tectronix, RM 565). Spikes were separated from background noise using a window discriminator (Mentor, N-570) and were counted with a digital counter (Hewlett-Packard, 5300B). When recording from the whole nerve, the window was set so that only the larger amplitude spikes, which were clearly separable from the background, were counted. All variables including the electroneurogram and discriminator signal were recorded on an ultra-violet light recorder (Honeywell, Visicorder, 1508). Vascular pressures were measured using strain gauge transducers (Statham, $P_{23_{Db}}$) and D.C. amplification.

With the carotid sinus open to the normal pulsatile arterial pressure, an electroneurogram (ENG) of activity (single fiber or whole nerve) was recorded for 15 sec. Then 1 of 3 solutions was injected into the sinus through the external carotid cannula: Ringer's solution, NE (10^{-6} M) or VP (vasopressin injection, synthetic, Parke-Davis, 12.5 μU/ml). An initial injection of 0.3 ml was made to fill the dead space in the cannula and stopcock, this was followed by a second injection of 0.3 ml. This volume was found not to cause overflow of the drug from the sinus, into the systemic circulation. After 10 sec a recording was taken over the next 15 sec. Between trials the sinus was washed with Ringer's solution and the animal's own blood.

To perfuse the sinus a clip was placed on the common carotid artery and intrasinus pressure controlled from the pressure bottle. The ENG was recorded whilst the pressure was held steady in steps between 2.6 kPa and 23.4 kPa. At each pressure the pressure bottle was temporarily clamped off, the clip on the carotid artery removed and the sinus injected with a test solution (0.3 ml). The carotid artery was reclamped and the connection to the pressure bottle restored. The ENG was recorded for 15 sec, 15 sec after restoring the controlled pressure. The solutions tested were again Ringer's solution, NE 10^{-6} M, VP 12.5 μU/ml. Comparisons of average numbers of nerve impulses/sec before and after drug administration were made using a Student's t-test for paired data. For the results from the per-

Impulse discharge (mean ± SE) from the whole sinus nerve or from single fibers during exposure of the sinus to normal pulsatile arterial pressure. Sinus filled with Ringer's solution, norepinephrine (NE, 10^{-6} M or 10^{-9} M) or vasopressin (VP, 12.5 μU/ml)

	Whole nerve (IMP/sec)	Single fibers
Ringer	421 ± 28	50.9 ± 13.7
NE, 10^{-6} M	426 ± 30	54.1 ± 11.7
	n = 10, $p < 0.005$	n = 6, n.s.
Ringer	330 ± 65	51.4 ± 13.4
VP, 12.5 μU/ml	348 ± 64	58.2 ± 12.9
	n = 7, $p < 0.005$	n = 6, $p < 0.01$

fused sinus, impulse discharge during drug administration was compared with that following the injection of Ringer's solution.

Results. Injection of 10^{-6} M NE or VP, 12.5 μU/ml into a carotid sinus exposed to the normal pulsatile arterial pressure caused an increase in discharge frequency in the recording from the whole carotid sinus nerve (table) with no change in systolic or diastolic pressure and no change in heart rate. Inspection of the electroneurogram showed both NE and VP caused increases in both large amplitude spikes and small amplitude spikes. Altering the window discriminator to selectively count populations of large or small amplitude spikes did not indicate any differences in the responses to NE or P. Injections of Ringer's solution had no effect on the discharge rate or pattern. A similar pattern was seen when recording from single fibers; NE, 10^{-6} M caused a small increase which was not statistically significant. Of the 6 fibers tested with 10^{-6} M NE, 5 increased their discharge and 1 decreased its discharge. Pitressin also caused a significant increase. Both NE and VP caused an increase in activity during both systole and the usually silent diastolic period. This was obvious either with the whole nerve or with single fibers.

When the carotid sinus was maintained at controlled pressures, NE 10^{-6} M increased impulse discharge at all intrasinus pressures between 5.2 kPa and 23.4 kPa when compared to injections of Ringer's solution. Statistically significant differences were observed at 15.6, 18.2 and 20.8 kPa (fig. 1). In the figures the rate of impulse discharge, which varied greatly between nerves, is expressed as a percentage of the maximum discharge observed with Ringer's solution in the sinus. Administration of VP 12.5 μU/ml also increased whole carotid sinus nerve activity. Significant increases in discharge frequency were seen at pressures of 10.4, 13.0, 15.6 and 20.8 kPa ($p < 0.05$). Examination of the ENG again indicated that there was an increase in the discharge from fibers of both large and small amplitude with both agents.

The results obtained for single fibers in a pressure controlled sinus were similar to those obtained for the whole nerve. NE 10^{-6} M caused a significant increase in discharge at all pressures between 10.4 and 20.8 kPa (fig. 2). VP 12.5 μU/ml also increased single fiber activity. These changes did not reach significance as 2 out of the 7 fibers tested decreased their discharge. The threshold pressure for firing for most fibers was 5.2–7.8 kPa. The frequency of discharge at threshold was 40–50 Hz. Two fibers in the NE group were silent at 7.8 kPa in Ringer's but were active at the same pressure with NE, 10^{-6} M. One fiber in the VP group was silent before and active after VP at 7.8 kPa. No systematic study of effects of the drugs on threshold was made.

Discussion. It is clear that both NE and VP increase the average frequency of discharge in the carotid sinus nerve. It was noted by Landgren et al.[4] that the increase in discharge in diastole could occur at relatively low sinus pressures and that was also apparent in our experiments. The distinction made by Landgren et al.[4] that VP activated both large and small amplitude discharge but that NE had a preferential effect on small amplitude discharge was not apparent in our experiments. The division of fibers by the amplitude of their potentials in a multifiber preparation is not reasonable since amplitude of potential depends not only on fiber size but also on interelectrode resistance and distance from the recording electrode. Recordings from single fibers confirmed the fact that both NE and VP increase the discharge during diastole in fibers normally firing mainly during the systolic rise in pressure. It was of interest to observe in a few single fibers that either NE or VP could cause a decrease in discharge frequency. Unfortunately this was not apparent at the time of the experiment and none of the fibers which decreased their discharge were tested with both agents. We were not able to demonstrate any differences between NE and VP in their effects on carotid sinus baroreceptor activity. When the effects of NE and VP were tested over a range of pressures in the carotid sinus effects were seen at all sinus pressures although statistical significance was usually demonstrated between 10.4 and 18.2 kPa. The fact that increases in discharge were seen above the maximum that could be achieved by increasing pressure to 23.4 kPa suggests that both drugs may act either by sensitizing or by causing contraction of smooth muscle in series with the receptor.

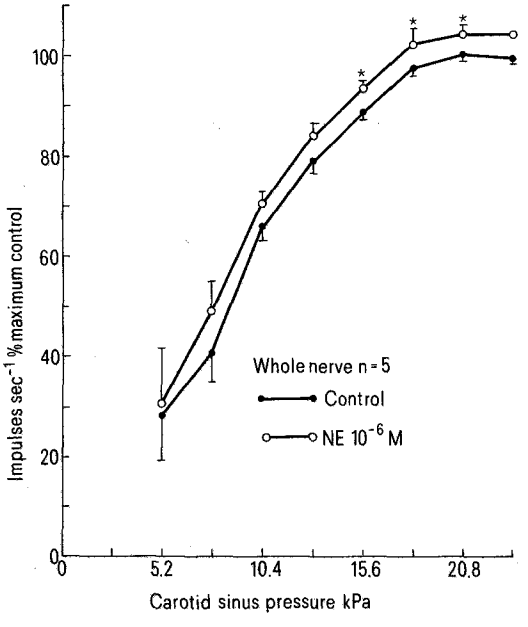

Figure 1. Impulse discharge in whole carotid sinus nerve, at different carotid sinus pressures, expressed as percentage of maximum control values. Control values (●) with Ringer's solution in the sinus, NE, 10^{-6} M (○). Values are means ± SE. Asterisks indicate the values with NE are significantly different ($p < 0.05$) from the control values.

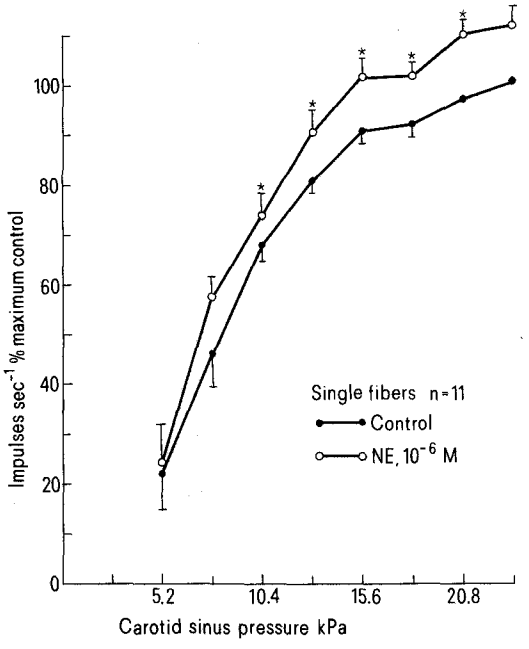

Figure 2. Impulse discharge in single functional fibers in the carotid sinus nerve at different carotid sinus pressures. Conventions as in figure 1.

Alteration of baroreceptor discharge following NE or VP administration may depend upon the relationship of the baroreceptor endings to smooth muscle cells within the adventitia[10]. The baroreceptor endings have been described as being located either in series or in parallel with the muscle elements. Single fiber recordings after either NE or VP showed that most fibers increased their discharge frequency either at normal pulsatile pressures or at perfused sinus pressures between 5.2 kPa and 23.4 kPa; presumably receptors located in series with contacting smooth muscle cells. A few single fibers showed a decreased discharge frequency with the sinus at normal pulsatile pressure and at all perfused sinus pressures; presumably located in parallel with smooth muscle cells. Similar results were obtained by Bergel et al.[8] in a few baroreceptor fibers in the dog. Since both NE and VP cause contraction of smooth muscle similar effects would be expected on nerve endings. Other workers[7] have suggested that 'sensitization' of the baroreceptor endings by NE could account for an increased baroreceptor discharge. This conclusion was based on the fact that 10^{-9} M NE increased discharge but 10^{6} M NE did not. They postulated that contraction of the vessel wall unloaded the baroreceptors in the presence of 10^{-6} M NE. If NE had sensitized receptors and VP had not, it may have been possible to show different effects. As we were unable to show any differences between NE and VP we must conclude either that both or neither substance causes sensitization. The fact that some fibers decreased their discharge to either NE or VP is consistent with the view that the receptor activity is modulated by smooth muscle activity rather than by sensitization of the receptors.

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Conformational flexibility of poly (dG-m^{5}dC) under very low salt conditions

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Summary. The methylated DNA polymer poly (dG-m^{5}dC) which exhibits a B helical conformation in solutions containing 20 mM NaCl, undergoes a gradual and reversible transition to the Z conformation as the NaCl concentration is lowered. The midpoint of this transition occurs around 5–6 mM NaCl. The conformational flexibility of this polymer at such low NaCl concentrations opens up the possibility of studying the effects of other perturbants with negligible interference from salt concentration effects.

The conformational transitions of the methylated polymer poly (dG-m^{5}dC) have received much attention in recent years, since the dinucleotide sequence dG-m^{5}dC occurs very frequently in eukaryotic DNA[1]. Possibilities of the correlation of such methylations with the control of genetic expression have been explored[2]. It has been shown by Behe and Felsenfeld[3] that the methylated polymer undergoes the B to Z transition very similar to the unmethylated polymer, but at much lower salt concentrations, with transition mid points occurring near 0.7 M NaCl or at 0.6 mM $MgCl_2$ in presence of 50 mM NACl, as opposed to the free polymer which has a transition mid point at 2.5 M NaCl or 0.7 $MgCl_2$[4]. These transitions of the methylated polymer have been recently confirmed by ^{31}P, ^{13}C and ^{1}H NMR studies[5–7] which indicate that the B to Z transition in the methylated polymer is complete at around 1.5 M NaCl. In the present study, we have detected a second B to Z transition of this methylated polymer at much lower NaCl concentrations below 20 mM in the absence of other cations. The transition is quite reversible in the range of 20 mM to 2 mM NaCl.

Materials and methods. The alternating double stranded polymer poly (dG-m^{5}dC) was purchased from P.L. Biochemicals. The polymer was homogeneous in CsCl density gradient with an $S_{20,w}$ of 8.5. Stock solutions of the polymer were prepared in 0.2 M NaCl solutions made from glass distilled, deionized water. The stock solutions ranged in pH from 6.8 to 7.0. Appropriate volumes of the stock solution were diluted freshly to obtain the required NaCl concentrations. Solid NaCl was added to gradually increase the concentration from 2 mM to 20 mM during the reversibility studies. All circular dichroic (CD) spectra were taken in a Jasco 10 Spectropolarimeter in a temperature controlled, jacketted cylindrical cell.

Results and discussion. Figure 1 represents the CD spectra of the methylated polymer at different NaCl concentrations ranging from 20 mM to 1 mM. At NaCl concentrations of 20 mM and above, this polymer exhibits a CD spectrum indicative of B helical conformation, with a broad positive band centering at 290 nm and a sharp negative band at 252 nm. As the concentration of NaCl is gradually lowered, an inversion of the CD spectrum takes place with a sharp negative band at 293 nm and a positive band at 276 nm. The variations of the CD ellipticities at different ionic strengths ranging from 2 mM to 30 mM NaCl are shown in figure 2. The negative ellipticity at 252 mM shows a sharp cooperative type of transition while those at 275 and 293 nm are rather broad and show biphasic tendencies. The transition midpoints in each case center around 5–6 mM NaCl. Under similar salt concentration conditions, the unmethylated polymer poly d(G-C) did not show any conformational transitions at room temperature and remained in the B helical conformation even at 2 mM NaCl[8].