A TECHNIQUE FOR THE QUANTITATIVE STUDY OF CAROTID SINUS BEHAVIOR

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THE study of input/output relationships of the carotid sinus is facilitated using digital computer techniques for analysis of recorded data. A three-stage computer program has been developed relating the timecourse of firing frequency of a single fiber of the carotid sinus nerve.

The first stage consists of generating an amplitude histogram from the direct input of action potentials. A Schmitt trigger is used to set a threshold level which an impulse must cross before it is recognized by the computer. On crossing the threshold an impulse triggers the computer at a rate of 50,000 samples/sec for 0.5 msec during which 25 samples are read into the computer. Only that portion of the action potential above the threshold is sampled. The rapid sampling rate insures accurate measurement of each peak amplitude which is then stored in the computer. After 1000 impulses have been sampled, an amplitude histogram is generated and displayed on a memory oscilloscope. Figure 1 is a direct read-out from the computer on the memory oscilloscope. The height of any point on this histogram indicated the number of peak amplitudes falling into that category. Because random noise is present on the recorded data, a multifiber preparation will result in a multimodal distribution as is seen in Fig. 1 which was taken using data with at least two fibers and the threshold set at a low level. The threshold was then raised and the same data was used to generate a new histogram. The unimodal distribution seen in Fig. 2 indicates that the fibers firing at lower amplitude level have been eliminated.

It is conceivable that on dissecting the nerve until only two or three active units remain that electrode summation still might occur. In this case, however, the distribution of the summed spikes will be evident as a distinct peak in the amplitude histogram. One can easily recognize such a peak in that it will be much smaller in height than the peak due to either single unit distribution.

The second stage of the program is designed to measure the frequency of action potentials from a single fiber as a function of time during each heart

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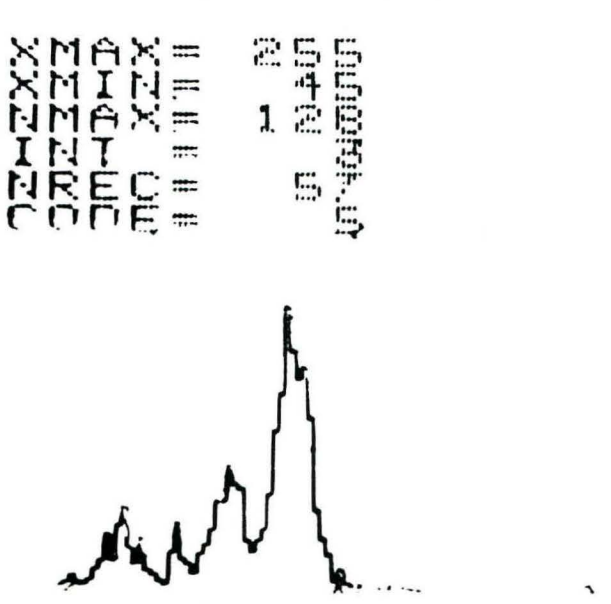

FIG. 1. Oscilloscope display of multifiber amplitude histogram.

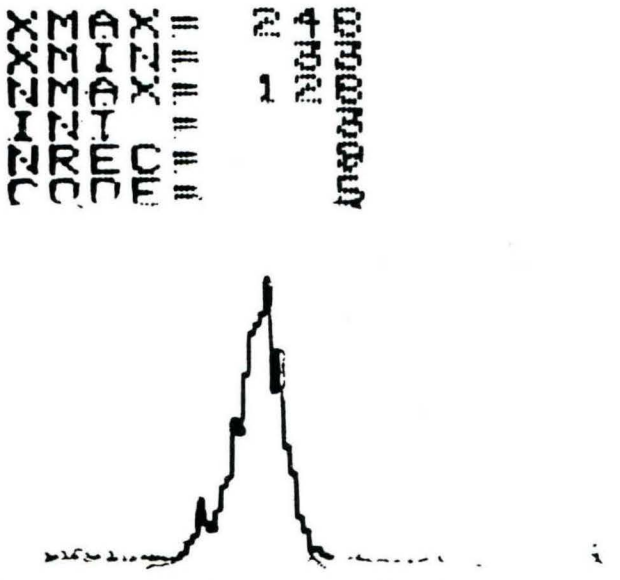

FIG. 2. Oscilloscope display of single unit amplitude histogram.

cycle. This is achieved by determining the interval between each pair of action potentials as it occurs. Figure 3 is a photograph of a face of an oscilloscope displaying the time-course of arterial pressure and frequency of firing of a single nerve fiber. In this program each action potential, upon crossing the threshold, interrupts the computer and causes the computer to read the time on its internal clock and that time is stored in memory. Upon occurrence of the following impulse above the present threshold, the clock is read again and the time lapse between impulses is determined. The frequency of action

FIG. 3. Continuous oscilloscope display from computer of carotid artery pressure (upper curve) and time-course of frequency of firing of single unit (lower curve).

potentials is measured as the reciprocal of this interspike interval and is stored in a frequency array in the computer. During the next heart cycle the frequency is again determined at each point during the cycle and the new frequency is averaged with the frequency at the corresponding point in the preceding heart cycles. In this way a beat-by-beat averaged frequency corresponding with each point in the heart cycle is established. Simultaneous sampling of the pressure pulse at a rate of 100 samples/sec is carried out. Each value 0.01 sec apart is averaged with the value occurring at the same time with the preceding heart cycle. These averaged values are stored in a pressure array in the computer. Two arrays have now been established, one containing the averaged frequencies, the other averaged pressures. These values are continuously displayed on an oscilloscope and in this way the development of the average waveform can be followed. When the frequency curve has become stable a manual interrupt will cause the averaged time-course for both pressure and frequency to be recorded onto digital tape for further analysis. The two arrays are replaced with zeros and the averaging is restarted. In this

way a series of records can be generated on digital tape for a variety of physiologic states for later analysis in phase three of the program.

This final phase consists of evaluation of the data by use of a model derived to predict the time-course of frequency of firing of the nerve with the time-course of pressure in the carotid sinus as the forcing function. From previous work two important facts about carotid sinus receptor mechanisms were known. These are represented by the equation at the top of Fig. 4. The first was the sensitivity of the receptors to the rate of change of pressure.[2] This is most evident during the positive rate of change of systole and less marked during the negative rate of change of diastole. The second fact was established by observing that nerve activity in response to a constant pressure input remains zero until a threshold pressure is exceeded and then the frequency of firing increases with increasing pressure.[3] The term P_t—P_{th} in Fig. 4 represents

$$F(t) = A\frac{dP^+}{dt} + B\frac{dP^-}{dt} + (P(t) - P_{th})C$$

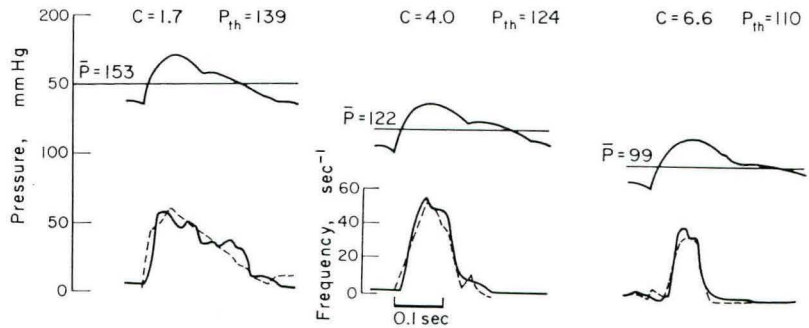

FIG. 4. Computer output of simple model-calculated frequency curves (dotted) and actual curves. Upper curves are carotid artery pressure.

this threshold phenomenon. When P_t is less than threshold pressure, no firing due to the threshold term is evident and firing of the nerve is due to the positive derivative term alone. When P_t is greater than threshold pressure, frequency of firing is influenced by both derivative and threshold terms. Successful experiments were performed on five mongrel dogs weighing 10 to 22 kg, anesthetized with nembutal 30 mg/kg. Positive pressure ventilation was maintained with room air during most of the experiments, although some recordings were made with the animal off the respirator in order to avoid the phasic variations in arterial pressure which occurred with the respiratory cycle. In each experiment, three physiologic pressure levels were produced. These consisted of control levels at the dog's normal pressure, elevated levels

introduced by an intravenous drip of norepinephrine in saline at a concentration of 2 mg of adrenalin hydrochloride in 500 cc of saline and decreased levels induced by intravenous drip of hexamethonium at a concentration to 50 mg of hexamethonium ion in 500 cc of saline.

In phase three of the program, the digitized data obtained from the first two parts is now used by the first approximation of the model described above to predict the frequency of firing of the carotid sinus nerve. In order to optimize model parameters so that the best correlation between frequency curves calculated by the model and the frequency curves obtained from the data easy communication of the investigator with the computer is necessary. This is facilitated by a peripheral station to the computer consisting of a memory oscilloscope and an octal coding switch.

On initialization of the program, a set of parameters used by the model in Fig. 4 are read into the computer from a card reader. After initialization all communication with the computer is through the oscilloscope and the four digit octal switch. Thus, two important aspects of model building are inherent in the system, the first being rapid and accurate calculations, the second, a direct link between the investigator and the model is possible.

Using this first approximation to the model as shown in Fig. 4, curves from each experiment, including all three physiologic states, were chosen and theoretical frequency curves were predicted. After each calculation by the model all three variables, namely, the input forcing function or pressure, the output predicted frequency curve and the actual frequency curve, were displayed on the memory oscilloscope. Here it was possible to visually review the results to see if a good correlation between predicted and measured frequency curves had been obtained. If not, then the model parameters were varied accordingly using the octal switch to input the parameter values and the curves were recalculated using the new parameters. In this way the effects of parameter variation on model prediction can be followed and new insight into the system obtained.

When the best fit possible has been obtained, the results are displayed on a printer along with all pertinent parameter values used to obtain the curve. As is seen in Fig. 4, a representation of one such experiment, good matches were obtained simply by varying the parameters C and P_{th}. Even over the small pressure range seen in this figure P_{th} varied from 139 mm Hg at high pressure levels to 110 mm Hg at lower pressure levels. C was seen to vary from 1.7 to 6.6 over the same pressure range in this experiment. Using the information obtained from the simple model, the derivation of a model which would predict the frequency of firing for a given experiment keeping the parameters constant in going from one physiologic state to another was determined. From the simple model another characteristic of the system was also observed; namely, that the effect of the positive derivative was more marked at low pressures and the negative derivative more marked at high pressures. Two

sensitivity terms were then included, one for the positive derivative and the other for the negative derivative. These were both made a function of mean pressure and can be seen as the coefficients of the derivative terms in Fig. 7.

It was also decided that a new threshold term was needed. To derive this term plots were made of C, the sensitivity ($\Delta f/\Delta P$) and threshold pressure

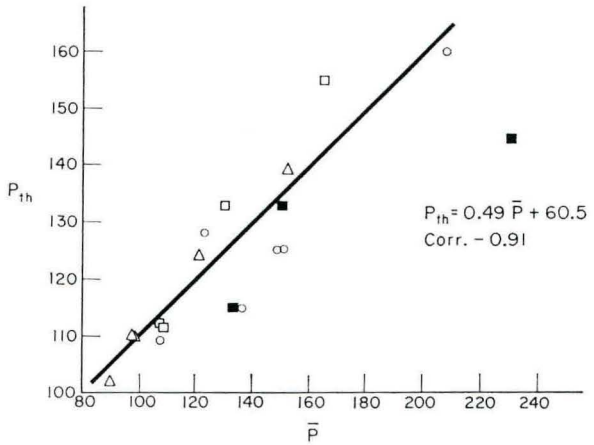

FIG. 5. Plot of threshold vs. mean pressure. Each different symbol represents one experiment.

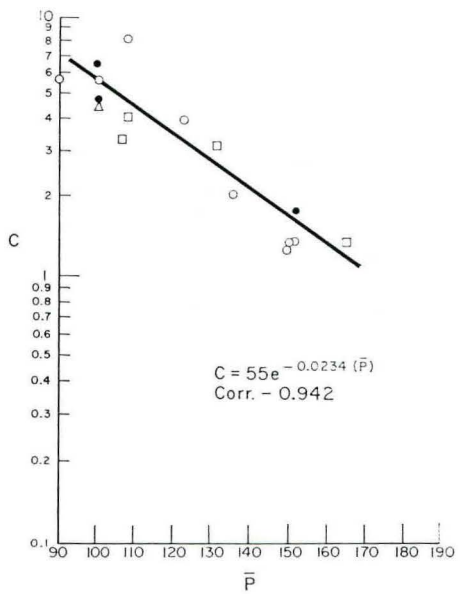

FIG. 6. Semi-logarithmic plot of sensitivity vs. mean pressure.

against mean pressure using the values of these terms as determined by the simple model in each experiment.

In Fig. 5 a plot of threshold pressure vs. mean pressure is shown. Threshold pressure was found to increase linearly with mean pressure and for every 2 mm Hg increase in mean pressure, the threshold pressure increases by 1 mm Hg. A regression analysis was used to determine the best fit of this line. The slope of the line is 0.49 and the intercept is 60.5 mm Hg.

Figure 6 shows a plot of the logarithm of C the static sensitivity, as a function of mean pressure for each experiment. Regression analysis showed that for every mm Hg increase in mean pressure, C increases exponentially by 2.34%. A new threshold term was now established as a function of mean pressure.

The model, which now included parameter values which varied with mean pressure, was used to predict the time-course of firing frequency over the whole range of physiological states. Figure 7 shows plots of the predicted and

$$F(t) = A(G - \bar{P})\frac{dP^{+}}{dt} + B(\bar{P} - H)\frac{dP^{-}}{dt} + C(P(t) - Co\bar{P} - Po)e^{-CK\bar{P}}$$

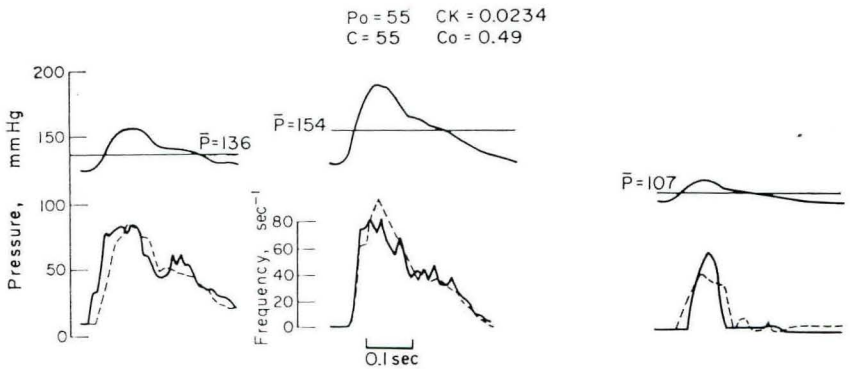

FIG. 7. Computer output of final model-calculated frequency curves (dotted) and actual curves.

actual frequency curves for one complete experiment using this model. The first curve represents the control state at a mean pressure of 154 mm Hg. The correlation is 0.94. The last curve is at decreased pressure produced by hexamethonium which lowered mean pressure to 107 mm Hg. The correlation is 0.89 using the same parameter values as in the control and hypertensive states.

Figure 8 shows the variation of proportional sensitivity with increasing mean pressure in six successful single fiber experiments on five dogs. Sensitivity C varied from 25 to 60 (sec mm Hg)$^{-1}$ while C_k was unchanged (0.0234) in

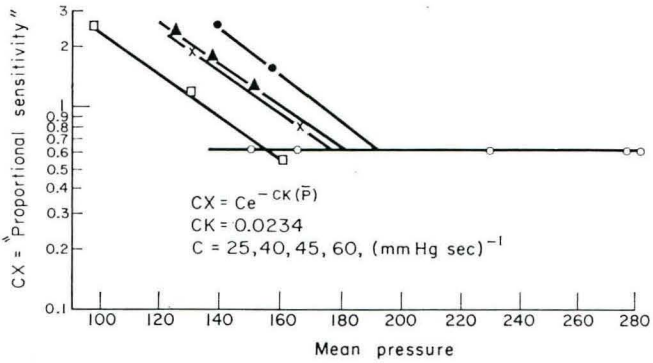

FIG. 8. Plot of proportional sensitivity vs. mean pressure. Horizontal line represents data from dog where two separate single units were obtained.

all four experiments. In the one dog where it was possible to record separately from two separate fibers the model parameters were the same for the two fibers but for both fibers the parameters differed significantly from those of the other dogs. In this dog mean pressure ranged from 151 to 280 mm Hg and the proportional sensitivity term *CX* did not change with increase in mean pressure as in the other dogs. This dog had an unusually high arterial mean pressure at the beginning of the experiment (150 mm Hg). No firing occurred below a mean pressure 140 mm Hg in this dog, while in the other animals activity was seen at much lower levels.

Figure 9 shows the plot of the static sensitivity against threshold. Notice that as threshold pressure increases with increase in mean pressure, sensitivity decreases.

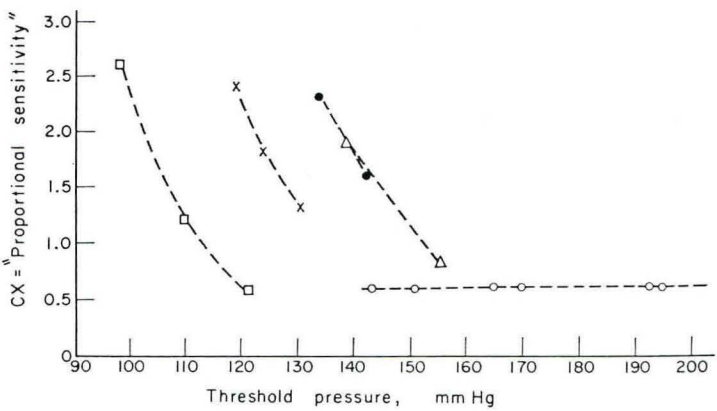

FIG. 9. Plot of proportional sensitivity vs. threshold pressure.

SUMMARY

Two important concepts have been considered in the study of the carotid sinus receptor mechanisms. The first is that the threshold for firing due to proportional term increases with increasing mean pressure down to a limit after which no change in sensitivity occurs.

A mathematical model has been described which predicts the time-course of frequency of action potentials on a single fiber of the carotid sinus nerve from the time-course of the carotid artery pressure. This system rapidly adapts to increase in mean pressure both by increasing the threshold and decreasing its sensitivity.

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DISCUSSION

SAGAWA: I wonder whether I understand you clearly. Did you say that one particular fiber exhibited both a fast adapting response component and a slow adapting proportional response component? Did you mean that the response depends not on the type of fiber, but the same fiber has two responding natures?

CHRISTENSEN: No. I meant that the threshold and sensitivity rapidly adapt to increase in mean pressure. This is not to say that there are two separate mechanisms in a receptor, one cannot conclude this.

TUCKMAN: Just one point. Did you say there was no firing under 140 mm Hg pressure?

CHRISTENSEN: This is true in one dog where I recorded separately from two fibers. At the beginning of the experiment the mean pressure was at 140 mm Hg. This was the control pressure of the dog at that time.

PETERSON: One of the things that each of the investigators could mention is, if the preparations were anesthetized and, if so, what anesthetic agent was used, because certainly we have found this is important in determining responses.

CHRISTENSEN: Nembutal was given to these dogs at 30 mg/kg.

WARNER: I really do not think that the anesthesia is important in studying the response of the organ itself. It is certainly of great importance when you are studying transfer functions through the central nervous system, but in our experience the level of anesthesia of the animal appears to play no role whatsoever in the transfer function of the organ itself.

PETERSON: I think this is something that can really be argued, because particularly with barbiturates the acid base levels change. This changes the behavior of both the myocardium and also the smooth muscle activity.

SCHER: I would like to get this clear again. Does this statement of adaptation of threshold mean that if one reaches the same pressure coming up and coming down, then maintains it, one will get a different firing level, i.e. that the threshold changes continuously as a result of the past history of pressure at the receptor? Are you saying the receptor is completely unstable?

PETERSON: Could I add to this, was the threshold determined by actual measurement of frequency of fibers, electrical activity of the fibers themselves?

CHRISTENSEN: On this threshold, I just say that it is rapidly adapting to mean pressure, that as the mean pressure rises the threshold rises rapidly, also, and as mean pressure decreases the threshold decreases.

CONWAY: I would like to draw Mr. Christensen out a little bit on this business of adaptation. I feel that a very essential point in the function of the baroreceptors is probably the rate of adaptation. He did not say how rapidly this occurred, and as this section appears to deal with the properties of the receptors, I wonder whether we could have some discussion in that area.

WARNER: In response to Dr. Conway's question I would like to say that in this particular study the rate of adaptation was not measured, but was in earlier studies which we reported using an analog computer. In the analog computer we averaged the response with varying time constants and found what time constant we had to put in to match the transistion from one state to another. Under these circumstances we found a time constant for the system of about seven seconds. This represents the rate of adaptation to the new mean pressure level.

PETERSON: This is adaptation of what to what?

WARNER: Adaptation of the constants we described in the preceding paper, that is, the sensitivity and the threshold are adapting at approximately the time constant of seven seconds.

PETERSON: This is not the adaptation of the receptors in the wall to mechanical changes, though?

WARNER: This is adaptation of the receptors in the wall to mechanical change, yes.

PETERSON: Seven seconds?

WARNER: That is right.

ZOTTERMAN: To zero?

WARNER: No. This is the time constant, which would be two-thirds of the response.

ALEXANDER: I would like to point out that this value is just about the viscoelastic time constant of the mechanics of the wall.

HEYMANS: I would say that it is adaptation not of the receptors but of the structures of the wall in which the receptors are located.

WARNER: Of course, the receptors are only important when considered as part of the context of the wall. So it is the system relating pressure to frequency of firing that is adapting.

HOMER R. WARNER:

I intend to approach this a little differently than Dr. Wang and I apologize because what I will say will be what I have tried to summarize in my own mind and is obviously going to be biased by the ideas I had prior to coming to this meeting.

Dr. Peterson has asked me to attempt to tie together in some kind of a concept, based on material presented here and what we may know, about the elements of the control loop that are outside of the carotid sinus and the central nervous system itself.

Let me first re-emphasize the concept I presented in my paper yesterday; namely, that the heart is a constant pressure pump. Let us look at this for a moment in terms of what it implies with regard to regulation of the circulation.

If the heart is a constant pressure pump (and by "constant" here I mean constant relative to some reference level that can be reset up and down) the regulation of flow through the system then becomes a matter of regulation at the local level.

The studies we heard today regarding exercise and regarding the effects of eating, could well be tied into such a hypothesis in which the needs for increased blood flow at the tissue level reflect themselves through increased end-products of metabolism through local vasodilatation at the local level to bring about an increase in flow.

The second point I would like to mention here concerns the relative roles of the vagus and sympathetic effects. We are all agreed, I think, that any action that the carotid sinus mechanism has will be affected on the efferent side through these two pathways. There has been considerable discussion back and forth in various types of experiments as to the relative importance of one or the other of these effector pathways. As I mentioned in a comment yesterday, an important clue in evaluating whether a reflex effect is being mediated through vagal or sympathetic pathways is the time-course of the response. We do have mathematical descriptions now of the heart rate response and the vasoconstrictor response to these efferent nerves. The sympathetic is characterized by a slow response to the onset of stimulation, or to a sudden increase in rate of stimulation. The time constant is in the neighborhood of 7–10 sec. The vagus response, on the other hand, often occurs within one heart beat and is extremely fast. And the time-course of these variables, I think, should be an important clue in helping us unscramble their relative roles under various complicated experimental conditions.

The off response to stimulation of these, again, is very different. The sympathetic heart rate response and the vasoconstrictor response often will take as long as 20 or 30 sec to return to its control level. The vagus off response does not occur quite as fast as the on response, but within a matter of 2 or 3 sec it will return to its control level.

Finally, I think the role of the carotid sinus in regulating cardiac output under a variety of physiologic states discussed in the reports we have heard today is consistent; that is, that the cardiac output responses to changes in carotid sinus activity are minimal. Although there are some effects on the force of contraction, both the studies in humans by Dr. Tuckman and the studies that I reported, showed pretty clearly that the carotid sinus mechanism has relatively little role in regulating cardiac output *per se* although it has a definite effect on heart rate. Variation in peripheral resistance is apparently the dominant mechanism by which arterial pressure is regulated through the carotid sinus mechanism.

PAUL KEZDI:

I was asked to discuss the meaning of what you heard during these two days and what has just been summarized by the previous speakers as it relates to the mechanism of hypertension.

I think we all agreed that hypertension can be considered a disease of regulation. We heard numerous papers and discussions yesterday and today regarding how the blood pressure control system works as a whole. Then we heard how the individual segments, the carotid baroreceptor system, the medullary centers, the effector side function, and the entire system can be tied together. We have scanty information in certain areas and more information in others.

We heard, and we agreed, that the baroreceptors are reset in hypertension. We do know that resetting occurs at the receptor site, but we do not know what role a change in the receptors themselves, and in the stiffness of the arterial wall play in this. There were indications that both are involved, but we do not know the degree of their participation. We also do not know whether resetting occurs in hypertension in the central nervous system. This is a difficult problem and very little work has been done to investigate central control of the baroreceptors in hypertension.

We also heard that there is evidence of "resetting" at the peripheral effector site, as Dr. McCubbin has shown to us. In the past we talked about increased vascular reactivity in hypertension as an inherent, probably hereditary defect. But we have seen that this can be produced by subpressor amounts of angiotensin. This is a form of resetting of the periphery. Now we see that there may be a whole chain of resetting mechanisms in hypertension. The entire blood pressure control loop is perhaps sensitized and the baroreceptors maintain the sympathetic output at a normal to increased level through resetting without which the angiotensin induced sympathetic potentiation could not be