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EFFECTS OF ELECTRICAL STIMULATION ON THE FREQUENCY OF CHEMORECEPTOR DISCHARGES

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The results presented in the preceding two papers indicate that chemoreceptor impulses in the carotid body are probably elicited by chemical action on the nerve endings. Acetylcholine (or an acetylcholine-like substance) seems to be released during moderate stimulation of the organ, induced by either hypoxia or hypercapnia. During intense chemoreceptor activity (produced by anoxia, interruption of flow or cyanide) other substances besides acetylcholine are probably released as well (Eyzaguirre & Koyano, 1965*a*, *b*).

The released substances may stimulate the sensory nerve endings and give rise to propagated action potentials in the chemosensory nerve fibres. Chemical release and stimulation of the sensory terminals suggest that carotid body chemoreceptors form a receptor synapse. This possibility has been already advanced by de Castro (1951) based on morphological observations. A synapse implies the presence of a presynaptic element, presumably formed in this case by the glomus cells, and a post-synaptic element, namely, the sensory nerve endings. If this is the case, one would expect that the chemoreceptor synapse may be activated by electrical stimulation of the presynaptic elements, since this occurs in effector junctions such as those in sympathetic ganglia and in muscle. Admittedly, the analogy may not be quite legitimate since the embryological origin of the carotid-body glomus cells has not been well defined (cf. Adams, 1958). Nonetheless, electrical stimulation of the carotid body was tried and results were quite striking, since the frequency of chemosensory discharges increased either after application of repetitive electrical pulses or during passage of direct currents through the carotid body. The sensory frequency changes were not due primarily and only to direct electrical effects on the sensory nerve endings. In fact, pharmacological tests showed that the electrically induced chemosensory discharge increase was probably due to release of a cholinergic substance from the glomus tissues. Partial and preliminary results have been published (Eyzaguirre & Koyano, 1963).

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METHODS

The carotid body was placed in a Perspex channel through which flowed Locke's solution, equilibrated with different gas mixtures at the desired pH and at the prevailing barometric pressure of 635–643 mm Hg. The organ was covered by a layer of paraffin oil (Eyzaguirre & Koyano, 1965*a*). Drugs were applied to the preparation, after dissolving the substances in the Locke's solution in the reservoir. The carotid nerve was lifted into the oil where filaments were prepared for stimulation and/or recording. Electrical stimulation of the carotid body was accomplished through a pair of platinum-iridium electrodes which were placed in the saline, one at each side of the organ. Usually, an earth electrode was placed at the oil-saline interface to decrease stimulus artifacts. Repetitive electrical pulses (0·1 msec in duration) at frequencies of from 1 to 100/sec and of various amplitudes were delivered from a Grass stimulator through a stepdown G.R. transformer. Direct currents (d.c.) were delivered by a constant-current-passing device which allowed delivery of currents through the wire stimulating electrodes for long periods of time (H. Fein, unpublished). The current intensity was carefully monitored on the oscilloscope screen. The frequency/sec of carotid nerve impulses was measured, sometimes, from photographic records obtained from the oscilloscope screen. More often, impulse frequency was measured by a counter-printer system once every 2 or every 12 sec, as shown previously (Eyzaguirre & Koyano, 1965*a*). During repetitive stimulation, counts obtained during stimulation were discarded when shock artifacts were appreciable. Consequently, most of the frequency curves shown in Results present the impulse frequency obtained before and after the application of repetitive stimulating pulses. When the glomus was stimulated by direct currents, the sensory impulse discharge was counted throughout the stimulating period since the shock artifact was only a transient one. The membrane potential of carotid body cells was measured with 3 M-KCl-filled micro-electrodes (30–80 MΩ a.c. resistance) connected to the oscilloscope d.c. amplifier via a cathode follower probe.

RESULTS

The results presented in this study were obtained from thirty-six experiments in which either repetitive square pulses or direct currents were employed to stimulate the preparation. The effects of different drugs (which directly or indirectly have been associated with cholinergic transmission in effector synapses) were tested on the electrically induced sensory discharge.

Effects of repetitive stimulation

Repeated stimuli at various frequencies were applied to the carotid body and the frequency of impulses was measured from the carotid nerve as indicated in Methods. When low-frequency pulses were applied (at 1/sec) there was a transient depression of the discharge. However, as time proceeded and stimulation continued there was a slow but clear increase in discharge frequency which either reached the base line or increased above this level. After stimulation ceased discharges returned slowly to base-line levels. At higher stimulating frequencies (4–100/sec), discharge depression occurred throughout the stimulation period. After interruption of stimulation there was a clear 'rebound' in sensory discharge frequency which increased to levels higher than the base line before returning to the

resting condition. This post-stimulation activation (PSA) was more pronounced at stimulating frequencies of about 20/sec than at higher or lower frequencies of stimulation.

The threshold for PSA varied in different preparations and, at times, it was clearly dependent on the orientation of the stimulating electrodes with regard to the glomus tissues. The magnitude of the phenomenon was directly dependent on the intensity of stimulation. The stimulus intensity, however, was never larger than that necessary to stimulate unmyelinated C fibres (cf. Eyzaguirre & Uchizono, 1961) for fear of damaging the nerve.

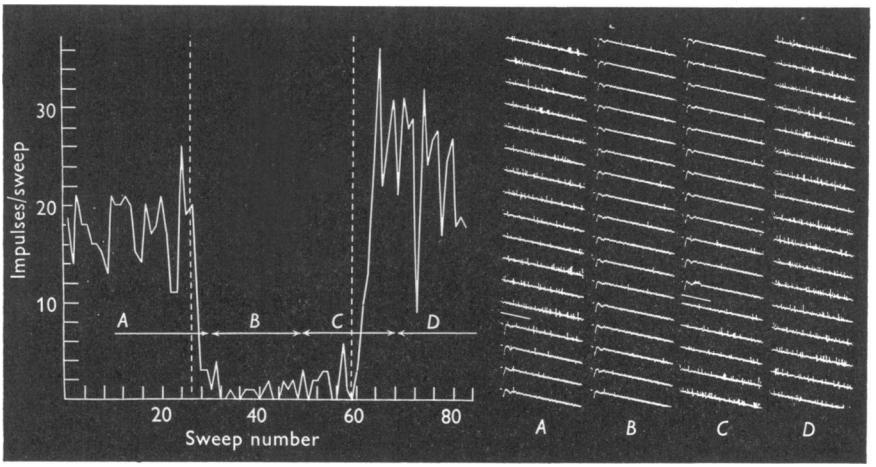

Fig. 1. Effects of electrical stimulation on chemosensory discharges of carotid body. Recording from large carotid nerve filament. Preparation bathed in Locke's solution equilibrated with 5 % CO_2 in O_2, pH 7·51 at 36° C. Flow, 2·0 ml./min. Right-hand side of illustration. Continuous sweeps recorded from oscilloscope; read from above downward and from left to right. Duration of each sweep, 240 msec. Near bottom of *A* beginning of carotid body repetitive stimulation at 4/sec and 12·5 V (horizontal bar). Stimulation interrupted near bottom of *C* (marked by horizontal bar). Notice increase in sensory discharge frequency at end of *C* and in *D*. Left-hand side of illustration. Sensory discharge curve constructed by counting impulses in each sweep. Between interrupted lines carotid body stimulation at 4/sec. Horizontal arrows labelled *A*, *B*, *C* and *D* show position of right-hand columns of same label from which discharge curve was constructed. Ordinate, number of impulses/sweep. Abscissa, sweep number. Further information in text.

Figure 1 illustrates an experiment in which the carotid body was stimulated by 12·5 V applied at 4/sec. The oscilloscope tracings presented at the right hand of the illustration show discharges obtained from a relatively large carotid nerve filament. *A*, *B*, *C* and *D* are continuous records which should be read from above downwards and from left to right. Near the bottom of *A* the horizontal bar indicates the onset of stimulation at

4/sec. The following records (bottom of *A*, *B* and part of *C*) show the nerve action potentials elicited by A and C fibre stimulation followed by a pause in the sensory discharges. At the horizontal bar in *C*, stimulation was interrupted and the discharge increased transiently above base-line levels. The left half of the illustration shows the number of impulses in each sweep plotted against the sweep number. Between the vertical interrupted lines the carotid body was stimulated at 4/sec. It may be seen that the sensory discharges declined to a very low value soon after the onset of stimulation. However, the sensory discharge started to recover before the end of stimulation. Once stimulation was interrupted, discharges increased rapidly to levels above the base line (PSA).

The experiment just described showed that during electrical stimulation of the carotid body the chemosensory discharge was depressed, although discharges started to increase in frequency before stimulation was interrupted. Discharge depression might be attributed to antidromic invasion of the nerve terminals by propagated impulses which may have produced hyperpolarization of the endings (cf. Eyzaguirre & Kuffler, 1955). However, recovery during stimulation and PSA (obtained after stimulation was discontinued) were not due entirely to membrane potential changes of the nerve endings. In fact, when the stimulating electrodes were moved up on to the nerve (the recording electrodes being placed on the nerve close to the carotid body), and the receptors stimulated by antidromic pulses at different frequencies, (i) there was a depression of the sensory discharges which remained unchanged for the duration of the stimulating period, and (ii) there was no increase in sensory discharge following antidromic nerve stimulation once the electrical pulses were discontinued (cf. also Fig. 3).

The magnitude of PSA was dependent on the intensity of the stimulating pulses as shown in the experiment illustrated in Fig. 2. As in Fig. 1, records should be read from above downwards and from left to right. In *A*, discharges obtained from a very fine carotid nerve filament (which contained only a few active units) are shown. At the bottom of *A* (marked by a dot), the carotid body was stimulated by pulses (7·5 V) delivered at 17/sec. Between *A* and *B*, 10 sec of recording is omitted. At the top of *B* (marked by dot), stimulation was interrupted. The frequency of the chemosensory discharges increased slowly, reaching a maximum in *D*, and started to decrease in *E*. After a period of rest the base-line discharge was recorded again (*F*) and the stimulating pulses were delivered again at 17/sec but this time the stimulus intensity was increased to 20 V. The onset of stimulation is also indicated by a dot at the bottom of *F*. Between *F* and *G*, 10 sec of recording is omitted. The end of stimulation is presented by a dot at the top of *G*. The increase in discharge, over base-line levels, is clearly seen in *G*, *H*, *I* and *J*.

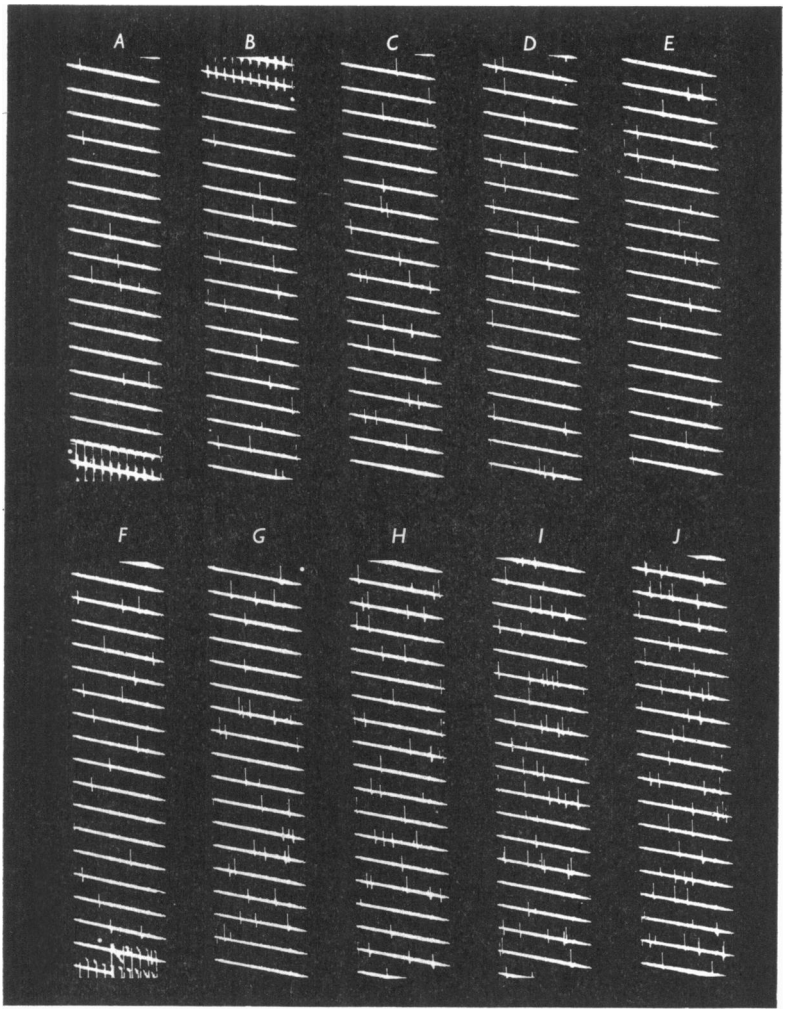

Fig. 2. Post-stimulation activation (PSA) after electrical stimulation of carotid body. Recording from very fine carotid nerve filament. Organ in Locke's solution equilibrated with 30 % O_2 in N_2, pH 7·36 at 36° C. Flow, 0·5 ml./min. *A–E* continuous records (read from above downward and from left to right), except for 10 sec omitted between *A* and *B*. At bottom of *A* (marked by dot) onset of stimulation with 7·5 V at 17/sec applied for 10 sec. At top of *B* (marked by dot) stimulation interrupted. Notice increase in discharge which reaches a peak in *D*. *F–J* same preparation. Read as in *A–E*. Same flow as in *A–E*. At bottom of *F* (marked by dot) beginning of stimulation with 20 V at 17/sec. Ten seconds recording omitted between *F* and *G*. At top of *G* end of stimulation (marked by dot). From *G* to *J* continuous records. Notice appreciable increase in sensory discharge frequency in *G–J*. In all cases, sweep duration 600 msec.

Site of origin of PSA

The carotid body is a complex organ in which structures, other than glomus cells and sensory endings of carotid nerve myelinated fibres, occur either within or around the carotid body capsule. Thus, de Castro (1926) has described the presence of nerve cells, some of which have presynaptic innervation provided by carotid nerve axons while others are innervated by fibres which originate in the superior cervical ganglion. The axons of nerve cells within the glomus innervate the local blood vessels (de Castro, 1926). In addition, the organ contains non-myelinated fibres of sympathetic origin (from the superior cervical ganglion) which traverse the organ and continue in the carotid nerve. Other non-myelinated fibres of non-sympathetic origin are found in the carotid nerve and seem to originate (or terminate) in the glomus (Eyzaguirre & Uchizono, 1961). Furthermore, the carotid body contains pressoreceptor endings whose fibres travel in the carotid nerve (de Castro, 1951). Therefore, it was necessary to design experiments in order to locate the site of origin of PSA. One of them is illustrated in Fig. 3.

The carotid body and its own nerve, in addition to a stretch of the ganglio-glomus nerve, were mounted in the Perspex channel through which flowed Locke's solution. Sensory discharges were recorded from the carotid nerve in oil (see Methods). Repetitive stimulating pulses were delivered to the carotid body as before. In *A*, PSA obtained after repetitive stimulation with 13·75 V applied at 10/sec for 25 sec is illustrated. When the stimulus strength was decreased to 3·75 V (record *B*), PSA was present although the change in sensory discharge frequency was less marked than in *A*. In *C* the recording electrodes were moved closer to the carotid body: one was dipped into the bathing Locke's solution while the other was placed on the nerve a few mm higher. The background frequency was reduced because of shorter inter-electrode distance. The stimulating electrodes were placed near the distal cut end of the nerve. Maximal repetitive pulses (also at 10/sec) were applied to the nerve for 25 sec and PSA did not occur. In *D*, the recording electrodes were placed again on the carotid nerve at the same place as had been used in *A* and *B*. The stimulating leads were placed on the stretch of ganglio-glomus nerve and stimulation at 10/sec for 25 sec applied again. The stimulus strength was enough to stimulate all fibres in the nerve. *D* shows that there was no increase in sensory discharge frequency over base-line levels after ganglio-glomus nerve stimulation. This observation confirms a previous one by Eyzaguirre & Lewin (1961).

The experiment illustrated in Fig. 3 shows that PSA was elicited only when the carotid body was stimulated and this effect was dependent on

the stimulus strength. PSA was not produced by stimulation of intraglomus nerve cell synapses, since stimulation of either the carotid nerve or the sympathetic ganglio-glomus nerve did not elicit such an effect. It was concluded, therefore, that PSA was elicited by either direct action of the repetitive pulses on sensory nerve endings (of either chemo- or presso-receptors) or through activation of the glomus cells.

In order to test whether or not electrical stimulation elicited PSA by direct action on the sensory nerve endings, the effects of repetitive stimu-

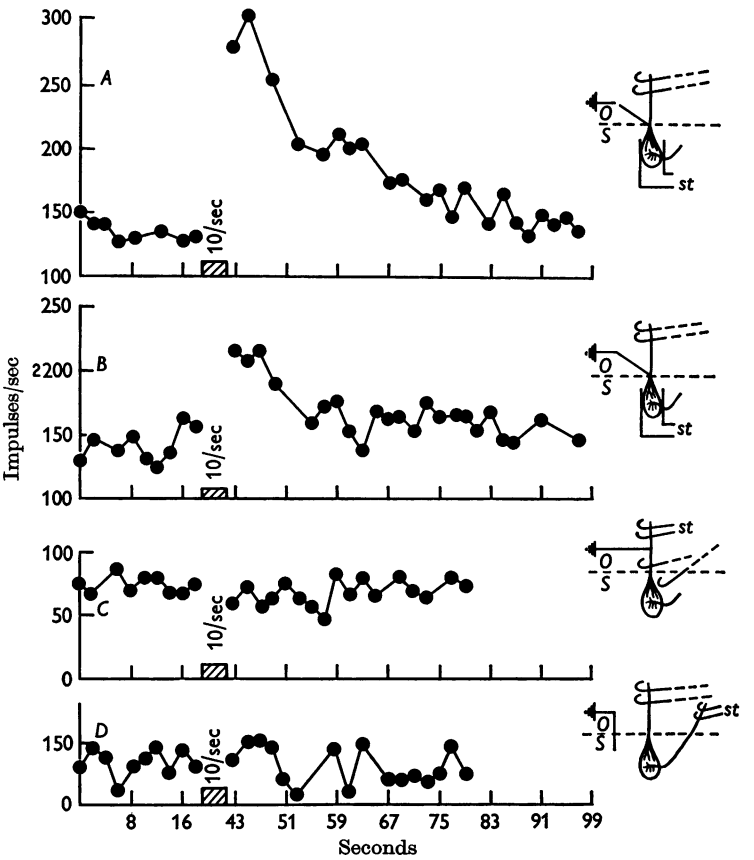

Fig. 3. Site of origin of PSA. Recording from whole carotid nerve. Carotid body in Perspex channel. Locke's solution equilibrated with 5 % CO_2 in O_2, pH 7·5 at 33° C, flowed at 3·5 ml./min. *A*, *B*, *C* and *D*, different experimental situations shown by drawings on right-hand side of illustration; *O*, oil; *S*, saline; *st*, stimulating electrodes. In all cases, pulses applied at 10/sec for 25 sec (striped bars). *A*, PSA elicited by carotid body stimulation with 13·75 V. *B*, as in *A*, but stimulus strength reduced to 3·75 V. *C*, maximal stimulation of carotid nerve. *D*, maximal stimulation of ganglio-glomus nerve. Ordinate, impulses/sec. Abscissa, time in sec for *A–D*.

lation on either carotid body chemoreceptor endings or pressoreceptor endings were compared. The latter were selected outside the capsule of the glomus. In either case, single fibre preparations were employed. Repetitive electrical stimulation of the carotid body elicited depression of single chemosensory fibre activity during stimulation, but a clear PSA developed soon after stimulation was withdrawn. Electrical stimulation of areas in which pressoreceptor endings were located elicited pressoreceptor discharge depression during stimulation but PSA did not occur. These experiments ruled out any participation of pressoreceptor activation in the development of PSA. Furthermore, the repetitive electrical pulses, as applied in this study, probably did not produce PSA through a direct action on chemoreceptor nerve endings. This conclusion is based both on the effects of saline flow on PSA (see immediately below) and on the assumption that both types of endings are similar except for differences in threshold to some forms of stimulation (cf. Eyzaguirre & Koyano, 1965*a*, *b*).

The second alternative explanation for the development of PSA, namely an action on the glomus cells, is that glomus cell activity stimulates the chemosensory terminals through liberation of a chemical and not through a process of current flow. To test this point, the effects of saline flow on PSA were studied, since a chemical effect should be more pronounced if flow was slow and vice versa. Figure 4 illustrates the effects of flow on the magnitude of PSA. In *A*, Locke's solution flowed at 1·0 ml./min and the base-line discharge was about 5/sec. Repetitive stimulating pulses at 20/sec were applied to the carotid body for 10 sec (striped bar) and the sensory discharge frequency increased to about 190/sec to decline slowly afterwards. In *B*, the flow of the bathing solution was increased to 2·2 ml./min and 4 min later repetitive pulses at the same frequency and intensity were applied again to the carotid body. The chemosensory discharge increased from a base line of about 2/sec to a peak of about 25/sec. Discharges declined to near base-line levels in about 150 sec. Effects of flow changes on this and other preparations (not illustrated) were frequently repeated with the same results: PSA was more marked when saline flow was slow. These effects were probably due to liberation of (a) chemical(s) which was swept away by the flowing saline. Slow flow probably tended to keep the substance (or substances) for a longer time around the nerve endings. One may argue, however, that slow flow leads to poor oxygenation of the tissues (Eyzaguirre & Koyano, 1965*a*) and that electrical stimulation of the carotid body increases oxygen consumption. This argument is not sustained by pharmacological effects on PSA (see later).

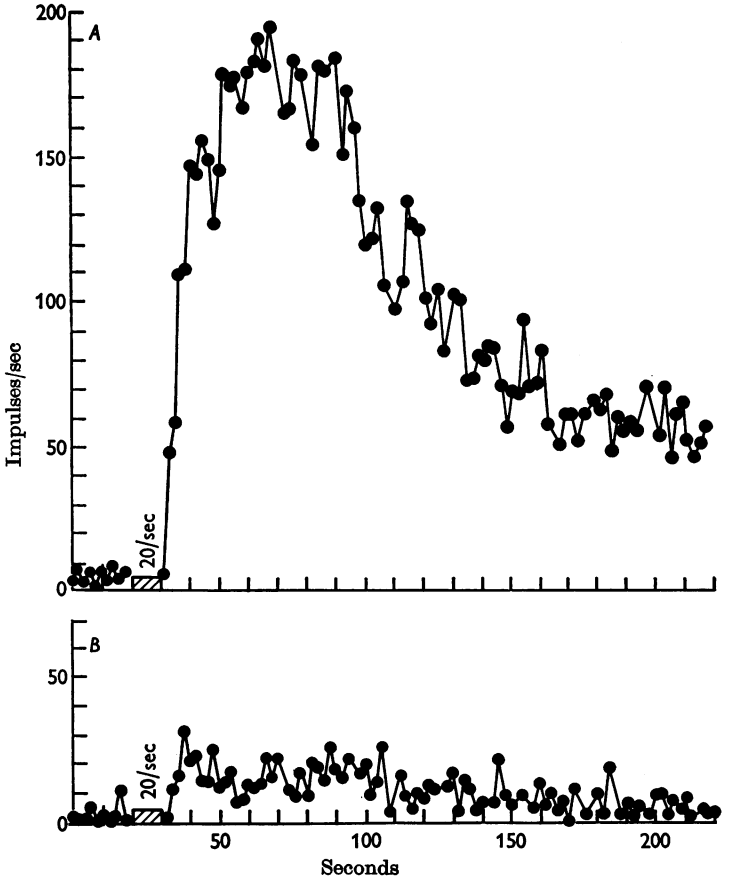

Fig. 4. Effects of saline flow on PSA. Recording from whole carotid nerve. Receptor bathed in Locke's solution equilibrated with 50 % O_2 in N_2, pH 7·38 at 36° C. In *A*, Locke's solution flowed at 1·0 ml./min and in *B* flow was 2·2 ml./min. In both cases, electrical pulses delivered to the carotid body at 20/sec for 10 sec (striped bars). Ordinates, impulses/sec. Abscissae, time in sec for *A* and *B*.

Magnitude of PSA in relation to base-line frequency

The previous study (Eyzaguirre & Koyano, 1965*b*) showed that the percentage increase in chemosensory discharge induced by acetylcholine was greater when the base-line frequency was lower and vice versa. This effect has been ascribed to combined effects of ACh and the normal chemical transmitter on the sensory nerve terminals.

In this study the magnitude of PSA was investigated in relation to the background frequency of the sensory discharges. The latter was varied by changing the oxygen concentration of the bathing saline. Flow and

temperature were maintained constant throughout the experiments. In each series, repetitive electrical pulses were delivered to the carotid body at constant pulse duration, amplitude and frequency. Such an experiment is illustrated in Fig. 5. The solid circles (linked by the solid line) represent the ratio of the average peak discharge frequency (obtained during PSA) to the base-line frequency when PSA was obtained by carotid body stimulation with 22·5 V at 20/sec for 10 sec. The open circles (linked by the broken line) represent the PSA-base-line frequency ratio obtained by carotid body stimulation with 7·5 V at the same frequency and for the same period of time in the same preparation.

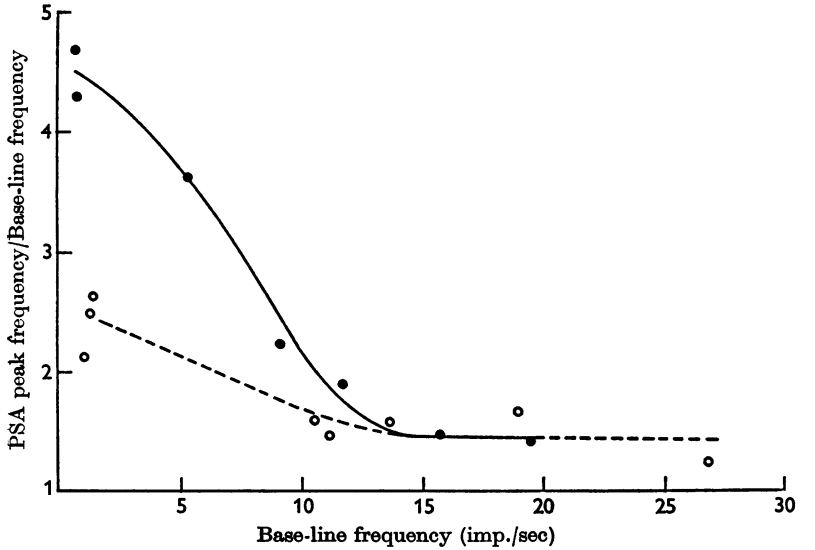

Fig. 5. PSA at different base-line frequencies. Locke's solution equilibrated with different oxygen mixtures (in N_2) to attain different base-line levels. Bathing solution (pH 7·36) flowed at 1·0 ml./min. Solid circles (linked by solid line) PSA-base-line frequency ratio obtained by stimulation of carotid body with 22·5 V at 20·sec for 10/sec. Open circles (linked by broken line) PSA-base-line frequency ratio obtained at stimulus intensity of 7·5 V. Ordinate, PSA-base-line frequency ratio. Abscissa, base-line frequency, impulses/sec.

The experiment just described shows that development of PSA was at least partly dependent on the base-line frequency: at lower background discharge PSA-base-line frequency ratio was greater than at higher frequencies of background discharge. As shown later, this characteristic had to be considered when the effects of different drugs on PSA were tested, particularly when such agents induced appreciable variations in the background discharge (cf. Eyzaguirre & Koyano, 1965*b*).

Effects of different agents on PSA

Effects of acetylcholine. The effects of ACh (Matheson, Coleman & Bell) on PSA were studied in a few preparations. Very small concentrations of ACh, presumably left after washing out a relatively strong solution of the drug, clearly potentiated PSA. Stronger ACh solution (10^{-6}, wt./vol.) did not have clear-cut effects on PSA. More interesting, perhaps, was the

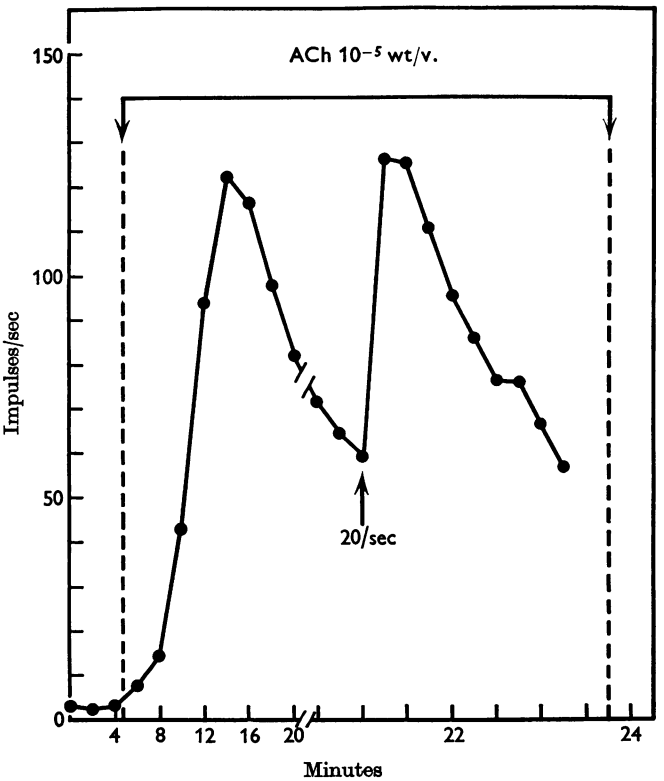

Fig. 6. Effects of electrical stimulation on the ACh response of carotid body. Recording from whole carotid nerve. Organ bathed in Locke's solution equilibrated with 30% O_2 in N_2, pH 7·37 at 33° C. Solution flowed at 0·6 ml./min. Between interrupted lines, application of 10^{-5} (wt./vol.) of ACh. At upward arrow, carotid body stimulated at 20/sec for 10 sec. Ordinate, impulses/sec. Abscissa, time in min. Notice change of time scale in abscissa.

effect of carotid body stimulation superimposed on a receptor already stimulated by ACh. This effect is shown in Fig. 6. Under normal Locke's solution the carotid nerve fibres discharged at about 3/sec. When ACh (10^{-5}, wt./vol.) was made to flow through the preparation, the discharge increased to a peak of 122/sec which declined to 60/sec after several

minutes. At this point the carotid body was stimulated at 20/sec for 10 sec. As soon as stimulation was withdrawn the sensory discharge increased to about 125/sec in a few minutes. This experiment indicates that the decline in sensory frequency, after an initial rise, observed during application of ACh is probably due to receptor adaptation. The increased discharge following electrical stimulation (PSA) may have been due to relief of adaptation provoked by application of a strong additional stimulus (cf. Eyzaguirre & Koyano, 1965*b*).

Effects of Ca and Mg ions. The effects of Ca and Mg ions were tested on PSA in order to determine whether or not this phenomenon was due to release of ACh (or a similar substance), since it was established that, in all probability, PSA was due to liberation of a chemical from the glomus tissues. In fact, it is known that an excess of Ca ions increases the ACh output in sympathetic ganglia while Mg has the opposite effect (Hutter & Kostial, 1954). Furthermore, while an excess of Ca^{2+} does not increase the magnitude of the end-plate potential at the neuromuscular junction (Kuffler, 1944), Mg^{2+} depresses neuromuscular transmission presumably owing to a reduction in ACh output. Mg block is relieved by Ca ions (del Castillo & Engbaek, 1954; Boyd & Martin, 1956).

When the carotid body responded to electrical stimulation with marked PSA, addition of $CaCl_2$ to the Locke's solution did not increase this phenomenon. However, when the preparation (bathed with normal Locke's solution) was relatively unresponsive to electrical stimulation, addition of $CaCl_2$ to the bathing solution almost invariably increased PSA. A representative experiment is illustrated in Fig. 7. In *A* the preparation was bathed with normal Locke's solution (2·16 mM-$CaCl_2$) and the base-line discharge was about 6/sec. The carotid body was stimulated with 10·0 V at 20/sec for 10 sec and the elicited PSA was very small. In *B*, the bathing solution was replaced with Locke which contained 4·32 m-mole/l. of $CaCl_2$. The background frequency was about 8/sec. Repetitive electrical stimulation (at the same voltage, frequency and for the same period of time) applied to the carotid body elicited a considerable PSA. After this series the bathing solutions were changed several times (from normal to $2 \times CaCl_2$ and vice versa) with similar results: high $[Ca^{2+}]$ induced PSA while normal $[Ca^{2+}]$ did not.

In other experiments (not illustrated) $[Ca^{2+}]$ was either reduced to one-half the normal amount or completely removed. When PSA clearly occurred in normal Locke's solution, reduction in the Ca^{2+} content of the solution only depressed this phenomenon. In a few instances, however, PSA did not occur either in normal Locke's solution or in a solution containing twice the normal concentration of $CaCl_2$. In those cases, reduction of the Ca^{2+} concentration to one-half induced the onset of PSA. In these

cases low-Ca^{2+} effects may have been due, in part, to increased excitability of the nerve endings (cf. Brink, 1954). But increased excitability alone did not seem to give rise to PSA since when Ca^{2+} was entirely removed (a situation in which nerve endings should have been even more excitable) PSA failed to develop.

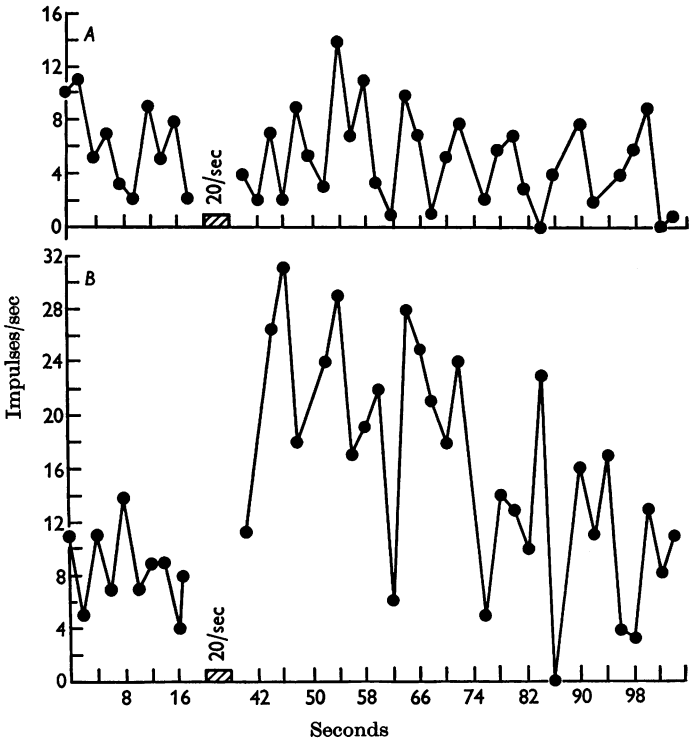

Fig. 7. Effects of Ca ions on PSA. Recording from whole carotid nerve. Glomus bathed in Locke's solution equilibrated with 50 % O_2 in N_2, pH 7·35 at 30° C. Flow 1·0 ml./min. In *A*, Locke's solution contained normal amount of $CaCl_2$ (2·16 m-mole/l.). During striped bar, carotid body stimulated with 10·0 V at 20/sec for 10 sec. Notice very small PSA. *B*, from same preparation taken 35 min after replacing normal Locke's solution with saline which contained twice the normal amount of $CaCl_2$ (4·32 m-mole/l.). Notice marked PSA obtained after stimulation of carotid body with 10·0 V at 20/sec for 10 sec. Ordinates, impulse/sec. Abscissae, time in sec for *A* and *B*.

The different results already described were probably due to different PSA thresholds in relation to the Ca^{2+} content. There is probably an optimal Ca^{2+} concentration for the onset of PSA and deviations from this ideal situation may tend to decrease or obliterate its appearance. For reasons still unknown the optimal concentration of Ca^{2+} necessary to elicit PSA varies in different preparations.

Mg ions employed in very small concentrations (less than 0·1 m-mole/l. $MgCl_2$) increased both the frequency of the background discharge and the magnitude of PSA. The reason for this potentiation is unknown, although it may be produced by some effects of the ion on the enzymatic systems of the preparation (cf. Eyzaguirre & Koyano, 1965*b*). Larger concentrations of $MgCl_2$ (from 2·5 to 5·0 m-mole/l.) either depressed or prevented the onset of PSA. This concentration of Mg^{2+} was less than that necessary to depress the chemoreceptor endings (cf. Eyzaguirre & Koyano, 1965*b*).

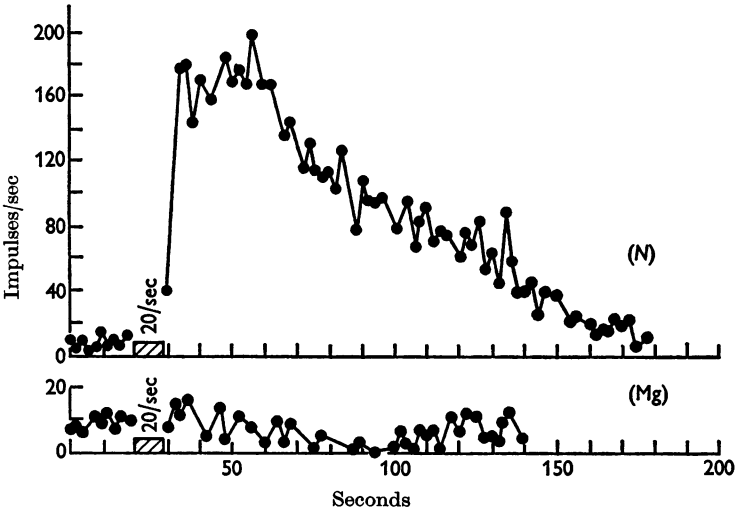

Fig. 8. Effects of Mg ions on PSA. Recording from whole carotid nerve. Receptor bathed in Locke's solution equilibrated with 30 % O_2 in N_2, pH 7·34 at 36·5° C. Saline flow, 1·2 ml./min. *N*, PSA obtained in normal Locke's solution after stimulation of carotid body at 20/sec for 10 sec (striped bar). Mg, block of PSA induced by 5·0 m-mole/l. $MgCl_2$. Ordinate, impulses/sec. Abscissa, time in sec for *N* and Mg.

A representative experiment is illustrated in Fig. 8. The carotid body was bathed with normal Locke's solution (*N*) equilibrated with 30 % O_2 in N_2. The receptor discharged at an average frequency of 7·3/sec. At the striped bar the carotid body was stimulated with 12·0 V applied at 20/sec for 10 sec. PSA was appreciable since the discharge reached an average peak of 180/sec to decline later to near base-line levels. After this experiment the receptor was bathed with Locke's solution which contained $MgCl_2$ 5·0 m-mole/l. (Mg). The base-line discharge was depressed and the oxygen concentration of the saline adjusted in order to bring this discharge to 9·1/sec which was near control levels. At this point the receptor was stimulated as before and PSA did not develop. The Mg-induced depression

was not relieved by addition of an excess of Ca ions (up to 4·32 m-mole/l. $CaCl_2$) or eserine (10^{-6}, wt./vol.).

Effects of cholinergic blocking agents. Several cholinergic blocking agents were tested on PSA. Atropine alkaloid (Nutritional Biochemical Corp.) in concentrations of 10^{-6} to 10^{-5} (wt./vol.), hexamethonium chloride (Matheson, Coleman & Bell) from 1 to 5×10^{-5} (wt./vol.) and D-tubocurarine HCl (Mann Research Labs.) from 10^{-6} to $1{\cdot}5 \times 10^{-5}$ (wt./vol.) were used. All these agents depressed PSA, although the intensity of their effects varied in different preparations depending on the dose and on the intensity and frequency of the stimulating pulses. In general, atropine seemed to be less effective than the other drugs.

Since the agents employed changed the base-line frequency (Eyzaguirre & Koyano, 1965*b*) it was necessary to run a series of control stimulations at different base-line levels while the receptor was bathed with normal Locke's solution. In order to achieve this, the oxygen concentration of the bathing saline was changed and, as the base line changed, stimulation was applied at different levels of background discharge. Then, a curve of PSA-base-line frequency ratio at different base-line frequencies was constructed. Once such a curve was obtained, the cholinergic blocking agent was added to the saline. One hour later, a new series of stimulations (at the same frequency, intensity and for the same period of time) was tried again at different base-line frequencies, also obtained by varying the oxygen concentration of the saline.

An experiment describing the effects of D-tubocurarine on PSA is presented in Fig. 9. The filled circles represent the PSA-base-line frequency ratio obtained during exposure of the organ to normal Locke's solution. The open circles show the ratio obtained after prolonged exposure of the carotid body to D-tubocurarine 10^{-5} (wt./vol.). It may be seen that when the sensory fibres discharged at more than 50/sec, D-tubocurarine did not change appreciably the response to electrical stimulation of the carotid body. However, when the base-line frequency was reduced to lower levels the curare curve showed a reduced PSA-base-line frequency ratio. Similar results were obtained with the other cholinergic blocking agents.

The described experiments show that cholinergic blocking agents may reduce PSA. However, this depression was partly relieved by increasing the stimulus strength. Also, cholinergic blocking agents depressed PSA even at doses which were too low to depress the base-line discharge, as shown in the preceding study (Eyzaguirre & Koyano, 1965*b*). Furthermore, PSA depression induced by the blocking agents was not relieved by addition of eserine (10^{-6}, wt./vol.) to the bathing solution.

Effects of eserine. The effects of eserine salicylate (10^{-6}, wt./vol.) on PSA were tried on preparations bathed in Locke's solution equilibrated with

either different concentrations of oxygen or 5–6 % CO_2 in O_2. Results were not constant since in some experiments the drug increased the amplitude of PSA, while in others it failed to do so. In other cases, eserine increased only the time course of PSA, while its amplitude remained unaltered. Finally, in some cases the drug-induced depression of PSA came back to control levels once eserine was washed out.

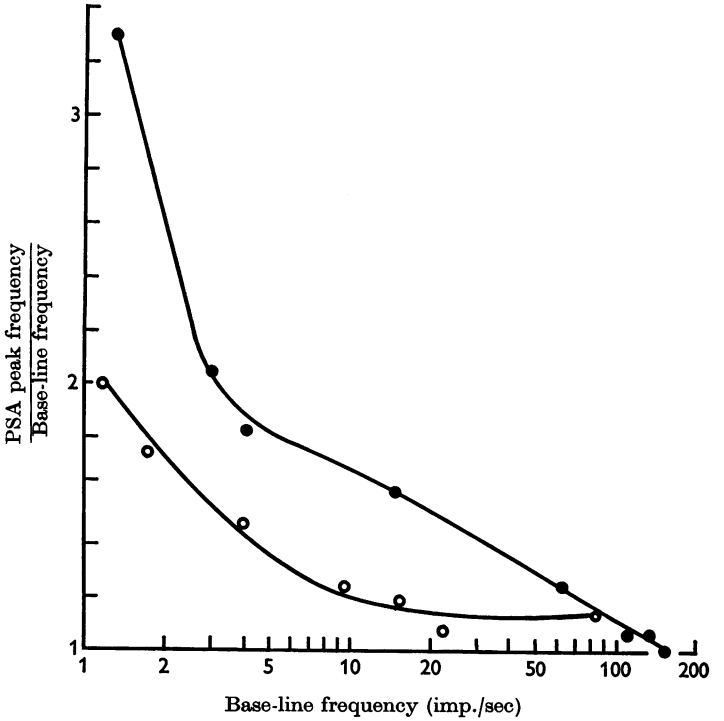

Fig. 9. Effect of D-tubocurarine on PSA. Recording from whole carotid nerve. Carotid body bathed in Locke's solution which flowed at 1·1–1·2 ml./min for 6 hr. pH 7·38 at 36·6° C. Saline equilibrated with gas mixtures containing different concentration of oxygen in nitrogen in order to change base-line frequency. Each point in curve, PSA-base-line frequency ratio obtained after stimulation of carotid body with 7·5 V applied at 20/sec for 10 sec. Filled circles, responses obtained in normal Locke's solution. Open circles, responses obtained after prolonged exposure of organ to D-tubocurarine 10^{-5} (wt./vol.).

Effects of CO_2. Occasionally, it was not possible to elicit PSA when the gas mixtures used to equilibrate the bathing Locke's solution did not contain CO_2. However, in the presence of this gas, electrical stimulation of the carotid body elicited PSA. The positive effects observed after electrical stimulation during exposure to CO_2 were not due to the level of background discharge since PSA occurred when the background discharge (produced by

CO_2) was intermediate between that obtained under different O_2 mixtures. There is no obvious explanation why CO_2 may favour the appearance of PSA. However, one may recall here that CO_2 also increases the response elicited by injected ACh and eserine and those effects have been assumed to be evoked by inactivation of tissue cholinesterase (Eyzaguirre & Koyano, 1965*b*).

Effects of direct currents

Direct currents were applied to the carotid body in saline while the nerve discharges were recorded in oil, as indicated in Methods. The current intensities ranged from 10 to 60μA and were generally applied for 60 sec. Direct currents had a striking effect on the frequency of the sensory discharges, the effect being dependent on the intensity and polarity of the stimulus. After many trials it was found that 20–40μA currents induced more marked effects than either higher or lower intensities of stimulation. The direction of current flow was also important. When current was passed in one direction (monitored as a 'negative' current) a marked increase in sensory discharge frequency was observed; this increased discharge returned slowly to base-line levels once stimulation was interrupted. When the direction of current flow was reversed (monitored as a 'positive' current) there was either no increase or depression of discharge frequency during stimulation. However, after 'positive' current stimulation was discontinued, the discharge frequency increased before returning to the base line. The direction of current flow probably acted here mainly through an effect on the nervous elements of the receptor and not because of a special orientation of the glomus tissues. It will be shown in the ensuing paper that polarity of stimulation applied to an upstream preparation did not have a significant effect on the electrically induced discharge of a downstream organ (see Eyzaguirre, Koyano & Taylor, 1965).

An experiment showing the effects of d.c. stimulation of the carotid body is shown in Fig. 10. The receptor discharged at about 3/sec (*A*). When 40μA 'negative' current was applied for 60 sec (striped bar) the discharge increased slowly to a peak of about 320/sec, 40 sec after the onset of stimulation. The discharge started to decline before stimulation was interrupted. After stimulation withdrawal discharges returned to base line in about 40 sec. After a period of 10 min (*B*) the receptor discharged at 15/sec. Direct current (40μA) was applied again but this time the polarity of the current was reversed ('positive' current. There was no increase in sensory discharge frequency during stimulation, However, when current passing was interrupted discharges increased to levels above 100/sec and after this peak they waned slowly toward the base line. The 'negative' current intensity used in this experiment was

too low to stimulate the nerve fibres directly. To do this the intensity had to be raised to 100 μA.

The results presented in Fig. 10 were probably due to release of a chemical substance from the tissues of the glomus and further evidence will be given in the following study with the use of carotid body preparations in series (Eyzaguirre *et al.* 1965). Direct currents did not act primarily and only on the sensory endings for the following reasons: (i) the

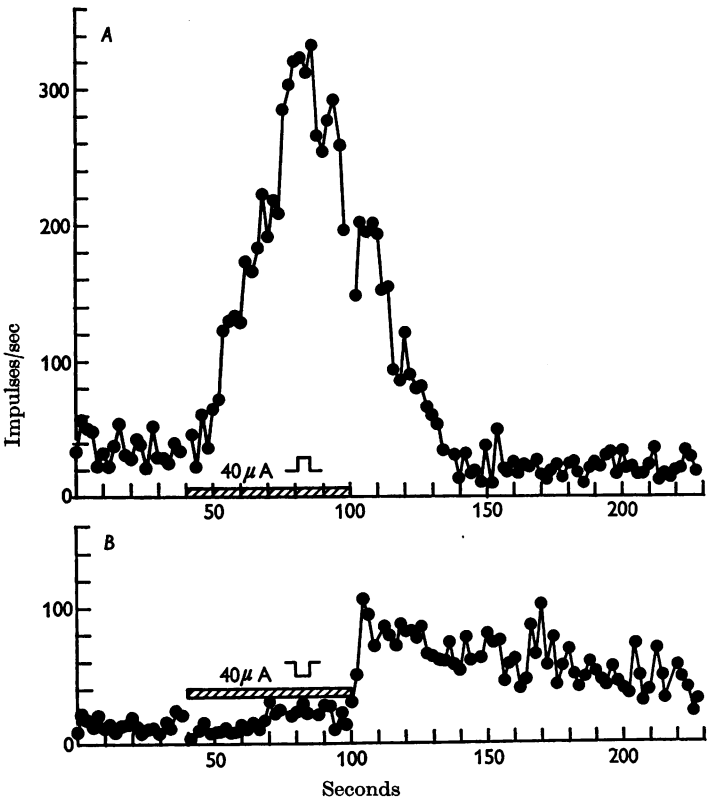

Fig. 10. Effects of direct currents on chemoreceptor discharge frequency. Recording from whole carotid nerve. Receptor bathed in Locke's solution equilibrated with 75 % O_2 in N_2, pH 7·36 at 36·3° C. Solution flowed at 0·3 ml./min. *A*, effect of 40 μA 'negative' current applied for 60 sec (striped bar). *B*, effect of 40 μA 'positive' current applied also for 60 sec (striped bar).

increased discharge observed in *A* was dependent on flow, being more marked when flow was slow; (ii) the discharge induced by 'negative' currents increased to a peak in 40 sec. One would expect that a direct action of current on the nerve endings would elicit either a steady discharge for the duration of the stimulation period (Edwards, 1955) or

a discharge reaching a peak soon after the onset of current flow followed by some adaptation; (iii) once stimulation was turned off the discharge did not come back abruptly to the base line but declined slowly; (iv) application of 20–60 μA short 'negative' current pulses (from 50 msec to 1 sec) did not elicit a train of sensory discharges, presumably because the stimulus intensity was below threshold. This was confirmed in single chemoreceptor fibre preparations where electrical events could be better analysed due to a lower background activity (not illustrated). One cannot deny, however, that direct currents (at the intensities employed here) had subthreshold effects on either the nerve endings or fibres. For instance, in *A*, the 'negative' current flow may have induced subthreshold depolarization of the nerve endings thus facilitating the sensory discharge increase. In *B*, the 'positive' currents may have not elicited an increased discharge during their application because hyperpolarization of the endings could occur. The 'rebound' phenomenon observed in *B* (after stimulation was interrupted) may have been due, at least in part, to a swing of the nerve ending membrane potential toward a depolarizing direction. More likely, however, this 'rebound' was probably produced by the presence of a long-lasting chemical effect which could not be observed during current passage due to block or depression of the nerve endings and/or fibres.

Effects of gallamine. Gallamine triethiodide (American Cyanamid Co.) in concentrations of 10^{-5} (wt./vol.) was the only cholinergic blocking agent employed in this series. The drug induced stimulation followed by depression of the chemosensory discharge frequency (Eyzaguirre & Koyano, 1965*b*). Consequently, after application of gallamine, the base line was adjusted (by changing the oxygen concentration of the saline) in order to evaluate the effects of the drug on the chemosensory frequency changes elicited by direct currents applied to the carotid body.

Gallamine depressed the sensory frequency curve elicited by direct currents as shown in Fig. 11. In *A*, the carotid body was immersed in normal Locke's solution (equilibrated with 50% O_2 in N_2) and discharges were recorded from a single chemosensory fibre. The fibre discharged at about 1·5/sec. At the striped bar 20 μA of negative current were passed through the carotid body for 60 sec. The discharge increased to a plateau of about 12/sec to decline to base-line levels about 100 sec after interruption of stimulation. In *B*, the carotid body had been exposed to gallamine (10^{-5}, wt./vol.) for 2 hr. At this point the base line was depressed (after an initial stimulation period), therefore the oxygen concentration of the saline was reduced to 30% O_2 in N_2 and the receptor fibre discharged at about 1/sec. Application of 20 μA of negative current elicited a discharge increase which was less than that of the control. After stimulation was

interrupted discharges returned to base-line levels relatively quickly. This and similar experiments showed that gallamine depressed the effect of d.c. stimulation on the chemosensory discharge. This effect was especially noticeable when curves showing direct current effects at different base-line frequencies were constructed. These curves were similar to those presented in Fig. 9, where PSA changes under D-tubocurarine were studied.

Effects of eserine. Eserine salycilate was applied to the preparation in concentrations of 10^{-6} (wt./vol.). The effects of the drug on the increased

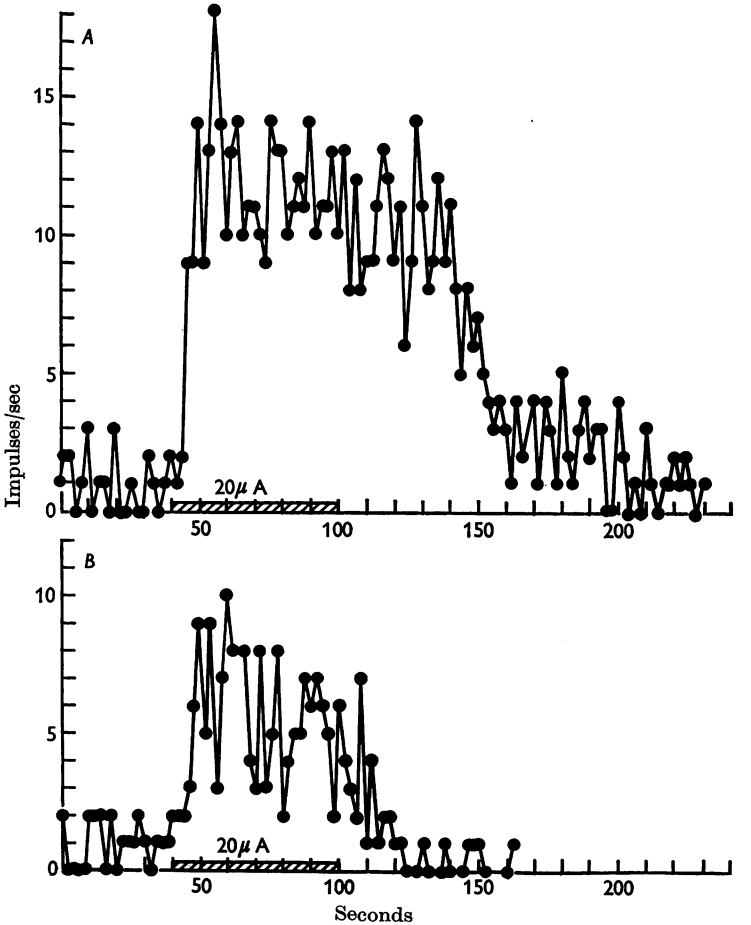

Fig. 11. Effect of gallamine on sensory frequency changes of single chemoreceptor fibre induced by direct 'negative' currents applied to carotid body. *A*, receptor bathed with Locke's solution equilibrated with 50 % O_2 in N_2, pH 7·36 at 36·3° C. Solution flowed at 0·3 ml./min. *B*, after 2 hr exposure of carotid body to 10^{-5} (wt./vol.) of gallamine. Locke's solution equilibrated this time with 30 % O_2 in N_2. In *A* and *B*, striped horizontal bars indicate period of current passage (20μA 'negative' current applied for 60 sec).

discharge elicited by direct currents were not marked. Eserine did not increase the amplitude of the frequency curve obtained during electrical stimulation of the carotid body, although at times it delayed the return of the discharge to the base line after stimulation withdrawal. In other cases, the same concentration of eserine did not change the time course of the return of the discharge rate to base-line levels. In all these instances, the base line was adjusted to near control levels (by changing the oxygen concentration of the saline) after application of eserine since the drug changed appreciably the background discharge of the receptor (cf. Eyzaguirre & Koyano, 1965*b*).

Lack of membrane potential changes during and after electrical stimulation of the carotid body

The carotid body, placed in Locke's solution, was impaled with high resistance micro-electrodes (see Methods) while the sensory discharges were recorded from the nerve previously lifted into the oil covering the preparation. It was assumed that the membrane potentials obtained (from 10 to 50 mV) were recorded from the glomus cells since they are numerous and larger than other cells in the carotid body (cf. Eyzaguirre & Lewin, 1961). Both repetitive stimulating pulses and direct currents were employed to stimulate the carotid body. Repetitive stimulation did not elicit membrane potential changes in the glomus cells either during stimulation or during development of PSA. Direct currents were applied at intensities of from 20 to 60 μA and the membrane potential of the glomus cells remained unchanged.

Lack of membrane potential changes during electrical stimulation may have been due to the fact that glomus cells are either spherical or ovoid, having diameters of from 6 to 10 μ (Ross, 1959). Under these conditions it is difficult to expect a net change in membrane potential since current flow through the carotid body would tend to depolarize one side of the cell while hyperpolarizing the other. However, it is interesting that after stimulation, when there was an increased discharge frequency, no membrane-potential changes were observed. These negative findings may indicate that appreciable K-ion movements probably did not occur. In fact, one would expect membrane depolarization if K ions moved out of the cell and became trapped outside the cell membrane. On the other hand, if K ions left the cell and were washed away by the flowing saline, one would expect membrane hyperpolarization.

DISCUSSION

The results presented in this paper show that electrical stimulation of the carotid body elicits an appreciable increase in sensory discharge frequency. When repeated electrical pulses were used there was depression of sensory discharge frequency during application of the stimuli, followed by increased activity after its withdrawal. Sensory discharge depression was probably due to antidromic invasion of the sensory nerve endings by the propagated action potentials elicited in the nerve. The increased sensory discharge frequency which followed interruption of stimulation (post-stimulation activation or PSA) was probably due to release of a chemical substance from the carotid body tissues which, in turn, activated the sensory nerve endings. The release of this substance probably began soon after the onset of stimulation, but its effects on the nerve terminals could not be detected until after stimulation withdrawal owing to the blocking effects of antidromic impulse invasion. In this respect it is interesting to notice that application of direct currents (when nerve action potentials were not elicited) produced a marked increase in chemoreceptor frequency which started to occur soon after the onset of stimulation. It is quite likely, therefore, that both repeated pulses and direct electrical currents increased chemosensory discharge frequency through a similar mechanism.

The released substance seemed to be of a cholinergic nature since electrical stimulation effects were directly dependent on the concentration of Ca^{2+}; they were depressed by Mg ions (5·0 m-mole/l. $MgCl_2$) and by cholinergic blocking agents. Eserine did not have very striking effects, although at times the drug prolonged the time course of the phenomenon. Furthermore, the electrically-induced phenomenon was sometimes potentiated by CO_2 and this gas also increased the effects of ACh and eserine on the chemoreceptor discharge presumably through an anticholinesterase effect (Eyzaguirre & Koyano, 1965*b*). The present experiments do not exclude the possibility that besides an ACh-like substance other substances may be released also during electrical stimulation of the carotid body. One of these might be of a catecholamine nature, although these agents are not very effective on the chemosensory discharge (Eyzaguirre & Koyano, 1965*b*). Another substance might be K ions, although intracellular recording experiments do not seem to favour this view.

The experiments reported in this paper show that carotid body chemoreceptors are quite unique and analogies with other known receptors are difficult to establish. The carotid body receptors seem to form a chemosensory 'synapse' (cf. de Castro, 1951), although electron microscopic studies have failed to reveal clear and specialized areas of contact between

the glomus cells and the sensory nerve endings. In effector synapses these areas are usually well defined. However, similarities in the behaviour of carotid body chemoreceptors and effector junctions (such as those in sympathetic ganglia and in muscle) seem to be present. In these effector junctions, ACh release, as the normal chemical transmitter, is likely to occur. Thus, post-tetanic potentiation observed in sympathetic ganglia, following repeated afferent stimulation (cf. Larrabee & Bronk, 1947), follows a behaviour which is similar to that observed in carotid body chemoreceptors after repeated glomus activation. In both cases the increment of activity (elicited by the stimuli) is proportionately greater when the background activity is lower and vice versa. With regard to the neuromuscular junction, similarities of behaviour between this junction and carotid body chemoreceptor synapses seem to exist (cf. Eyzaguirre & Koyano, 1965*a*). In this study the following analogies have been established: (i) the frequency of miniature end-plate potentials (which are probably produced by liberation of small ACh packets (cf. Katz, 1962)) is increased by application of subthreshold currents to the motor nerve endings (del Castillo & Katz, 1954); the frequency of the chemosensory discharges also increases during d.c. stimulation of the carotid body, an effect which is not due to direct stimulation of the sensory terminals; (ii) the effectiveness of neuromuscular transmission varies directly with the Ca^{2+} concentration and inversely with the Mg^{2+} concentration of the environment (del Castillo & Engbaek, 1954; Boyd & Martin, 1956). In our experiments the electrically induced chemosensory discharge is also directly dependent on Ca^{2+} and inversely dependent on Mg^{2+}; (iii) cholinergic blocking agents depress both neuromuscular transmission (cf. Katz, 1962) and chemoreceptor activity induced by electrical stimulation.

The following paper will show that ACh is indeed present in carotid body tissues (Eyzaguirre *et al.* 1965); therefore, it is conceivable that this substance may be released during electrical stimulation of the carotid body as the results presented in this paper seem to indicate.

SUMMARY

1. The carotid body was stimulated by repeated electrical pulses and the effects on sensory discharge frequency were analysed. Electrical stimulation induced depression of the sensory discharge frequency during stimulation, but after withdrawal of stimulation the sensory discharge increased above base-line levels (post-stimulation activation or PSA). PSA was directly related to the intensity and frequency of the stimulating pulses. The phenomenon appeared only when the carotid body was stimulated, being absent when a similar type of stimulation was applied to either the carotid nerve or the ganglio-glomus nerve.

2. PSA was more marked when the flow of the bathing solution was slow. The phenomenon was directly dependent on the Ca^{2+} concentration of the environment. Mg ions depressed PSA. Ganglionic blocking agents such as hexamethonium and D-tubocurarine clearly depressed this phenomenon while atropine was less effective. Eserine salicylate was relatively ineffective in changing the amplitude of PSA, although sometimes it prolonged its time course.

3. Application of direct currents to the carotid body elicited a marked increase in sensory discharge frequency during current passage. This effect depended on the polarity, intensity and duration of the stimulating current. Like PSA these d.c. induced effects were dependent on flow; the effect was depressed by gallamine and sometimes it was prolonged by eserine.

4. Neither repeated pulses nor direct currents changed the membrane potential of glomus cells.

5. It is concluded that PSA and the electrically induced chemosensory frequency increase elicited by direct currents are similar. Both seem to be produced by release of a cholinergic agent from the glomus tissues which, in turn, activates the sensory nerve endings.

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