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THE RESPONSE OF THE CHEMICAL RECEPTORS OF THE CAROTID SINUS TO THE TENSION OF CO_2 IN THE ARTERIAL BLOOD IN THE CAT.[1]

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Introduction.

In a previous paper Bogue and Stella [1935] described in the carotid sinus nerve a fresh type of afferent impulse appearing in asphyxia and anoxæmia, and having no relation with the endosinusal pressure. These impulses were found to originate from end organs other than the stretch receptors studied by Bronk [1931], and by Bronk and Stella [1932; 1935]. They would in fact persist in preparations where all the stretch receptors had apparently been severed from their connections with the nerve. Also the type of the action potentials was slightly different from that of the pressure impulses; and finally their distinction from the latter is further shown by the fact that asphyxia and anoxæmia do not excite the stretch receptors at all [Bronk and Stella, 1935].

It seemed very probable that this fresh type of impulse might represent the discharge of special chemical receptors, such as must exist in the carotid sinus (or more exactly in the carotid body, according to Heymans and Bouckaert [1932]), to account for the powerful circulatory and respiratory reflexes elicited from this region in response to comparatively moderate variations of the respiratory conditions of the arterial blood [Heymans, Bouckaert and Dautrebande, 1930; Owen and Gesell, 1931; Schmidt, 1932; Selladurai and Wright, 1932; Bernthal, 1934; Heymans, Bouckaert and Samaan, 1935; Gayet,

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Bennati and Quivy, 1935, and others]. According to some workers the susceptibility of this region to carbon dioxide is so great that even at normal tensions a tonic state of moderate reflex excitation of the respiratory centres is maintained [Selladurai and Wright, 1932; Witt, Katz and Kohn, 1934; Stella, 1935].

It has been found in the present investigation that a "chemical" discharge in the nerve of Hering, much smaller than that recorded by Bogue and Stella during asphyxia and anoxæmia, is present even under normal conditions of respiration. It is apparently kept up by the CO_2 or hydrogen-ion concentration of normal blood. The relation between the intensity of this discharge and the tension of CO_2 in the arterial blood has also been studied.

Methods.

The experiments were performed on cats anæsthetized with chloralose (0·07 g. per kg. intraperitoneally) and urethane (0·08 g. per kg. subcutaneously). The action potentials were recorded from the peripheral stump of the nerve of Hering, cut at its origin from the glossopharyngeal. The usual technique, with a resistance-capacity amplifier and a Matthews' oscillograph, was employed.

In view of the fact that the stretch receptors would not be affected by the chemical conditions to be studied here [Bronk and Stella, 1935], the peripheral distribution of the nerve was in some cases left untouched, *i.e.* the stretch receptors were not denervated. In the majority of the experiments, however, the denervation technique described by Bogue and Stella [1935] was used, and the nerve left connected only with chemical receptors. This makes the analysis of the records easier, and eliminates all effects of possible fluctuation of the general blood-pressure.

In some few instances this rather difficult technique was found unnecessary; *i.e.* when the chemical fibres were found to be all running in a separate small trunk, parallel to the main nerve, and like the latter joining the glossopharyngeal. In most cases the chest was open and the animal kept under artificial respiration. When the effect of the tension of carbon dioxide was to be investigated, the amount of pulmonary ventilation was maintained constant and at a high level throughout, and mixtures of air and varying percentages of the gas tried. An adequate supply of oxygen was thus ensured, while the tension of carbon dioxide in the arterial blood was determined in samples drawn from the femoral artery and analysed by the method of van Slyke.

The effects of interrupting and re-establishing the blood circulation in the carotid sinus have also been studied; for this purpose the left subclavian artery was tied at its origin, and a loop prepared round the innominate artery. This could then be temporarily pulled, and the artery occluded. By this method a complete interruption of the blood supply to the region was reasonably ensured, while, on the other hand, on releasing the artery, free circulation of pure arterial blood would be quickly restored. The latter condition was important when trying to determine the time taken by the asphyxial excitation to subside.

Results.

Threshold tension of CO_2.

During natural breathing a considerable discharge was as a rule present, completely unaffected by changes of the arterial pressure. Accumulation of carbon dioxide in the arterial blood resulting from inefficient pulmonary ventilation of the animal under the anæsthetic was no doubt one of the causes. But a moderate discharge was also noticed in cases in which, owing to a light degree of anæsthesia, the respiratory activity seemed very little impaired. Anyway, when artificial respiration was applied, the discharge was usually completely abolished only if the ventilation was sufficient to lower the tension of carbon dioxide in the arterial blood to levels below the normal. Thus at a tension of that gas as low as 33–35 mm. Hg, a very small discharge consisting of a few sporadic impulses was usually still present, and disappeared when the pulmonary ventilation was further increased.

Not all the chemical receptors of this region seem, however, to possess the same low threshold. Thus if a continuous record is made while the CO_2 in the blood is being washed out, and the intensity of the discharge gradually diminishing, it appears that the number of active fibres is steadily diminishing; and before the whole nerve becomes completely quiescent, one or perhaps two fibres will be seen to maintain an irregular sporadic activity over a considerable range of diminishing CO_2 tension. Exactly the reverse is noticed when the artificial respiration is gradually diminished and the chemical discharge allowed to build up slowly. A similar variability of the threshold value in different end organs has also been noticed among the stretch receptors of the carotid sinus [Bronk and Stella, 1932; 1935] and seems in fact to represent one of the features of end organs generally. It was interesting to see it repeated also in the case of this fresh type of endings.

Relation between the tension of carbon dioxide in the arterial blood, and the intensity of the "chemical" discharge.

The following experiments will show, even better than those described above, the actual importance of carbon dioxide as a factor which can bring the chemical receptors of the sinus into activity. The animals were kept under a constant degree of artificial respiration, while various mixtures of carbon dioxide and air were substituted for air so as to increase the tension of that gas in the arterial blood. The artificial respiration was so adjusted that, while inhaling pure air, no chemical discharge or only a very moderate one would be present. The percentage of CO_2 in the mixtures varied between 3 and 7. The tension of oxygen

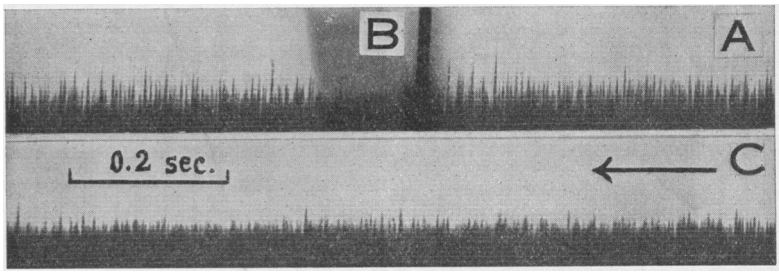

Fig. 1. Action currents from the peripheral end of a branch of the carotid sinus nerve showing chemical discharge independent of endosinusal pressure. Cat under artificial respiration and with chest open; left subclavian artery tied. *A*, before, and *B*, 3 sec. after complete occlusion of the innominate artery. *C*, artificial respiration increased; circulation uninterrupted. In this and all subsequent records time is in 0·2 sec.; all records are to be read from right to left.

therefore even during inhalation of the highest percentages of CO_2 was comparatively very little diminished. This point was of course important, in view of the stimulating effect of anoxæmia [Bogue and Stella, 1934; 1935].

The results obtained in this series of experiments are well illustrated in Figs. 1 and 2 taken from a typical experiment. The "chemical" fibres were all running in a separate nerve trunk containing no pressure fibres. Therefore, any manipulation likely to cause damage to the carotid sinus, or carotid body, was avoided.

The absence of pressure fibres and the complete independence of the observed discharges from the probable effects of the endosinusal pressure were established in this and similar experiments by the following observations: first, it was always possible to abolish the discharge and make the nerve silent by simply increasing the amount of the pulmonary ventilation.

At this stage, moreover, pulling on the common carotid was tried, a procedure which is known to excite the stretch receptors considerably; but no trace was noticed of fresh activity. Second, if while the nerve was very active, on account of the respiratory conditions of the blood, the

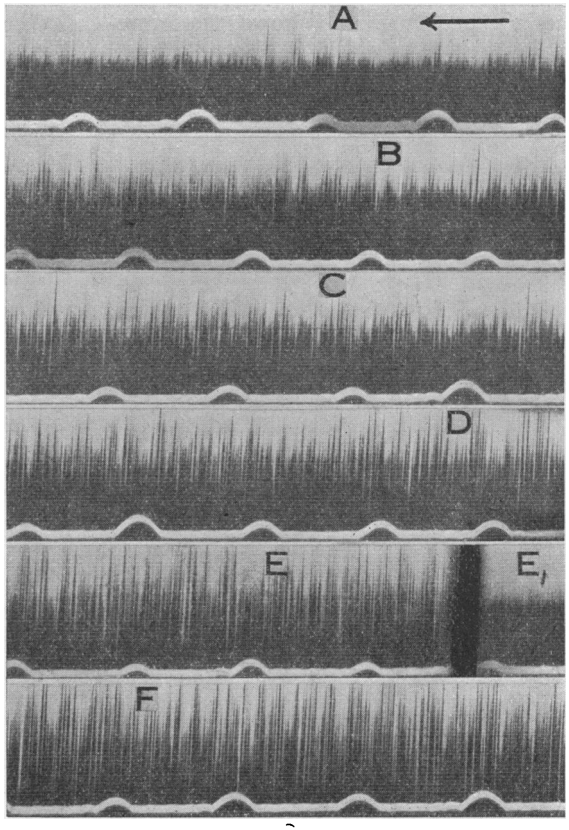

Fig. 2. Shows the dependence of the intensity of the discharge upon the respiratory conditions of the blood. *A*, the CO_2 tension in the arterial blood is 35 mm. Hg; a moderate discharge of a few impulses is already visible; *B*, CO_2 tension is 50 mm. Hg; *C*, 59 mm. Hg; *D*, 72 mm. Hg; E_1, hyperventilation with air; *E*, nitrogen is substituted for air; *F*, 5 p.c. CO_2 in nitrogen is inhaled.

pressure in the carotid sinus was suddenly dropped to the lowest possible levels, the nerve discharge did not seem to undergo the slightest alteration.

Fig. 1 illustrates such results as these. Under a moderate degree of artificial ventilation with air the fall of endosinusal pressure from 160 mm. Hg, down to about 10 mm. Hg, produced by occluding the innominate

artery, has no influence upon the discharge (compare *A* and *B*, Fig. 1). On the other hand, the washing out of CO_2 by increasing the artificial ventilation is followed by a distinct reduction in the intensity of the discharge (*C*, Fig. 1). *B* was taken 3 sec. after the innominate artery had been occluded. At this time there was no circulation of blood in the carotid body. The figure also shows, therefore, that the circulation of blood *per se*, *i.e.* apart from the supply and removal of chemical substances, has no influence upon the activity of the chemical receptors.

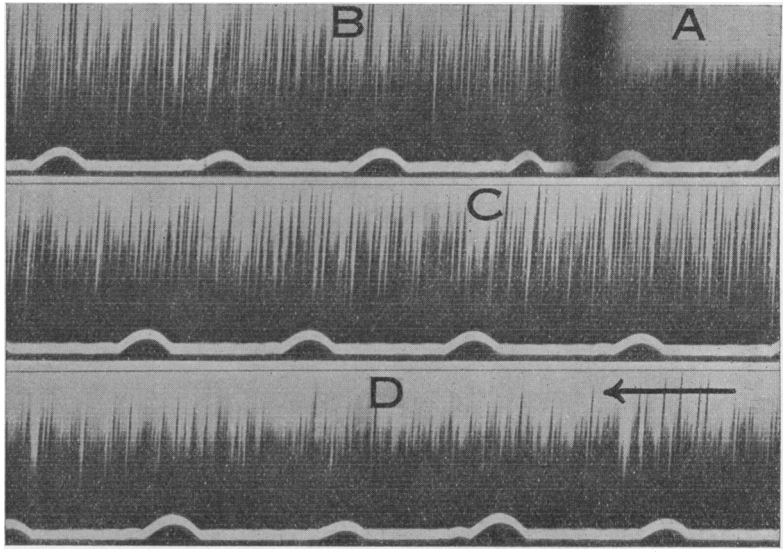

Fig. 3. The effect of stopping and re-starting the artificial respiration. Cat with chest open. *A*, respiration on; *B*, 30 sec. after stopping the artificial ventilation; *C*, immediately after re-starting the respiration pump; *D*, at the end of the second stroke of the pump. Variations of the endosinusal pressure have no effect on this discharge.

Fig. 2 shows the discharge in its relation to the tension of carbon dioxide in the arterial blood. It will be seen that starting from a level just below the normal and extending to a tension of about 70 mm. Hg, the discharge steadily increases in its intensity (*A*, *B*, *C* and *D*). The process is reversible, *i.e.* the same effects are obtained by going in the reverse direction from the higher to the lower tensions.

These records and the corresponding arterial blood samples were taken on each occasion after the fresh carbon dioxide mixture had been inhaled sufficiently long to allow the tension of that gas in the arterial blood to reach a reasonably steady state.

The increase in the nerve activity, however, was seen to follow very early after the inhalation of higher carbon dioxide mixtures had begun; the very first signs of it were in fact noticeable within a little more than a second. It will be pointed out below that this is due to the very short latent period of the end organs concerned. But since the increase of carbon dioxide tension under the present conditions was found to take place in the blood very gradually, the early response of the receptors must also be taken as an indication of their high sensitivity towards comparatively small variations in the intensity of this stimulus. Tensions of carbon dioxide higher than the one given in Fig. 2 *D* were not tried, but it is presumable that the effect would then have been even greater. The figure also shows the marked effect of acute anoxæmia, *E*, and of inhalation of a mixture of 5 p.c. CO_2 in nitrogen, *F*, as in fact would have been expected from the greater intensity of the reflex excitation of respiration which would occur in the last two cases.

Fig. 3 shows how a very considerable discharge, brought about by interrupting the artificial respiration for 30 sec., quickly diminished on re-starting the respiration pump. *C* was taken while the first stroke of the pump was going on; *D* immediately after the second stroke, and the intensity of the discharge is seen to be already reduced to less than one-half.

Latent period of the chemical receptors.

No accurate measurement has been made of the time elapsing before the chemical receptors begin to respond to a given new tension of carbon dioxide. The present experimental conditions would not allow such a determination; it has been possible, however, to obtain a rough idea on the point by considering the rapidity with which an increased percentage of that gas in the inhaled air was followed by the first indication of greater activity in the nerve. Having regard also to the time the blood must have taken to travel from the lungs to the carotid sinus, it may be safely assumed that the latent period is of the order of a fraction of a second. The rapidity of these end organs to respond to an increased carbon dioxide tension was particularly well noticeable when a mixture of that gas and air was inhaled by an animal in which, owing to hyperventilation, the nerve until then had been completely silent.

The subsidence of the excitation after removal of the stimulus also occurs early. This is well seen in the example given in Fig. 4; the very marked discharge at *B* was caused by prolonged interruption of the blood supply to the carotid sinus [Bogue and Stella, 1935] (closure of the innominate artery and left subclavian); at the signal X the innominate

artery was released, and the effect of asphyxia is seen to disappear in about four-fifths of a second. If the fully arterial blood in this experiment had been able to reach the end organs immediately, it is presumable that the subsidence of the discharge would have occurred even more quickly. In this case the excitation was due to anoxæmia as well as to accumulation of carbon dioxide in the tissues, but it seems justifiable to assume that the results would not differ essentially in the case of stimulation from carbon dioxide only.

Adaptation.

It has already been pointed out by Bogue and Stella [1935] that the discharge of the chemical receptors ensuing upon complete interruption of the circulation of blood in the carotid sinus can be seen to continue undiminished even after as long as 30 min. The course of the stimulating

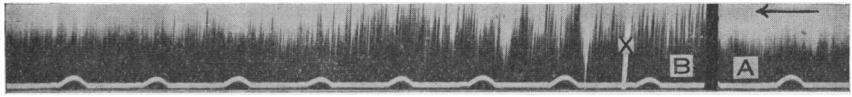

Fig. 4. Shows a large discharge provoked by prolonged interruption of circulation in the carotid sinus and its rapid subsidence on restoring the circulation. Chest open and artificial respiration on; left subclavian artery tied. *A*, circulation in the carotid sinus is normal; *B*, record taken 1 min. after complete occlusion of the innominate artery. During the record, at X, the artery is released; the discharge comes down to its original intensity after about 1 sec.

condition or conditions in these cases was not known, and it was very likely continually increasing during the whole time. We made therefore some observations on the behaviour of discharge in response to a given constant tension of carbon dioxide when maintained over long stretches of time (12–15 min.).

Mixtures of 3 and 5 p.c. CO_2 in air were used (artificial respiration), and, after allowing the blood gases to reach their new equilibrium, records of the discharge were taken every 2–3 min.

So far as can be judged from these records where so many active fibres were present, we have been unable to detect any sure sign in the sense of a progressive diminution in the intensity of the discharge itself. We think that the degree of adaptation of these end organs is very small indeed. It is possible of course that some adaptation would take place early, *i.e.* within the first moments after the application of the stimulus if this could be applied suddenly.

Discussion.

The evidence obtained in this investigation shows that the "chemical" discharge in the carotid sinus nerve is originating in endings which are specifically sensitive to the chemical conditions of the blood. Under adequate supply of oxygen their activity is strictly dependent on the tension of CO_2 in the arterial blood. Some of these endings begin to discharge at tensions of this gas as low as 33–35 mm. Hg. As the tension is gradually raised, the intensity of the discharge rapidly increases to reach nearly a maximal value at tensions of 80–85 mm. Hg. It may be pointed out that the whole range of increasing activity of these receptors lies well within the range of physiological and physio-pathological conditions of the blood, at which values, moreover, all other end organs so far studied in muscles, lungs, etc., remain quite unaffected. Carbon dioxide, or probably the hydrogen concentration of the blood, represents therefore an adequate physiological stimulus for these receptors.

The variations in the activity of these receptors in response to the various conditions tried are seen, moreover, to take place in the same direction as would the corresponding variations of the respiratory activity if the carotid sinus nerves were not cut [Heymans, Bouckaert and Dautrebande, 1930, etc.]. It may be mentioned in this respect that the reflex stimulating action of carbon dioxide on respiration, which had been denied by Gollwitzer-Meier and Schulte [1931] and by Mies [1932], has very recently been confirmed by Gayet, Bennati and Quivy [1935]. These last authors find, moreover, that such action can be quite marked already at tensions of that gas within physiological limits. It seems justifiable, therefore, to regard the "chemical" discharge as the afferent augmentor message in the mechanism of these reflexes. We have also observed that a discharge, similar to those seen under very high tensions of CO_2, or severe asphyxia or anoxæmia, is elicited by local application in the carotid sinus of nicotine or cyanide in small doses such as are known to provoke marked reflex excitation of respiration [Heymans, Bouckaert and Regniers, 1933]. The very small degree of adaptation of the receptors concerned is in further support of their importance in the reflex regulation of respiration.

It is also very probable that the continuous moderate discharge of these impulses, noticeable even under physiological conditions of the blood, is responsible for a tonic excitation of the respiratory centres, such as is claimed by Selladurai and Wright [1932], Witt, Katz and Kohn [1934], and by one of us [Stella, 1935]. According

to these workers, double denervation of the carotid sinus is, under certain circumstances, followed by a varying degree of respiratory depression.

Perfusion experiments of Heymans, Bouckaert and Dautrebande [1930] which showed that, if acapnic is substituted for normal or hypercapnic blood, there follows a temporary, but marked, inhibition of respiration, might perhaps have been interpreted as indicating that acapnic and indeed also alkaline blood may cause a fresh discharge of inhibitory impulses as against the augmentor ones above described. To test this possibility, various degrees of acapnia were tried in some of the present experiments, by varying the magnitude of the artificial ventilation. Below the threshold tension of carbon dioxide, however, never was there observed any sign of fresh activity in the nerve, no matter how far the hyperventilation was pushed. The inhibitory effect described by the authors mentioned was presumably due to sudden cessation of the augmentor discharge.

Bogue and Stella [1935] have described the building-up of a powerful chemical discharge in animals suddenly killed while in good condition of pulmonary ventilation. This occurrence, which was taken to be due to asphyxia of the end organs, was confirmed in the present research, in the case of temporary occlusion of the left subclavian and innominate arteries. The intensity of the discharge thus obtained reaches in about half a minute the highest values as yet recorded in this nerve. We believe that a discharge of similar origin was mainly responsible for the marked stimulation of respiration observed by Schmidt [1932] when the pump, perfusing the isolated carotid sinus, was temporarily arrested. Schmidt was investigating the difference in the inhibitory effect upon respiration of pulsatile pressure in the carotid sinus as compared with constant pressure. He noticed that when the mean pressure was very low, cessation of pulsation by stopping the pump was followed in the cat by a great excitation of the respiratory activity, and even sometimes by generalised convulsions. On re-starting the pump, powerful inhibition of respiration was noticed, sometimes leading to temporary but complete apnœa. In view of the results described in the present paper, the most reasonable interpretation of these dramatic findings seems to be that, owing to the low and non-pulsatile pressure, the chemical receptors in the carotid body were not being properly supplied with blood, and were in a state of marked asphyxial excitation.

SUMMARY.

1. The "chemical" discharge in the carotid sinus nerve, recorded by Bogue and Stella in anoxæmic or asphyxiated cats, is shown here to be present also under the usual conditions of respiration, only its intensity is markedly reduced.

2. It originates from receptors specifically sensitive to the respiratory conditions of the blood.

3. At tensions of CO_2 in the arterial blood at or below 32–35 mm. Hg all these receptors are at rest. Above that level they begin to discharge, and the intensity of the latter is the greater the higher the tension of the gas.

4. The threshold tension of CO_2 is not the same for all receptors, and it is probable that some of them are thrown into activity only at high tensions of that gas, or perhaps only in acute anoxæmia or asphyxia.

5. The "chemical" receptors seem to possess a very small degree of adaptation, if any.

6. The response to variations of CO_2 tension in the arterial blood is very prompt.

We wish to take this opportunity to express our sincere thanks to Prof. C. Lovatt Evans for his kind and helpful interest in the present work.

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