VI. THE PROBLEM OF TRANSMISSION AT CHEMORECEPTORS

TRANSMISSION AT CHEMORECEPTORS

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The idea of de Castro (9) that the carotid body is a chemoreceptive organ, has been definitively proven by the discovery of Heymans, Bouckaert and Dautrebande (16) that it is stimulated to increased activity by oxygen want as well as by accumulation of carbon dioxide. With regard to the mechanism of stimulation Winder (31) observed that a local poisoning of the carotid segments with monoiodoacetic acid could be obtained such that the response of the chemoreceptors to anoxia was abolished, whereas it remained unaltered or weakened to hypercapnia. Since monoiodoacetic acid suppresses anaerobic glycolysis and the formation of intermediary or final acid products of glycolysis, these results suggested a causal relationship between intracellular acidity and response. Bernthal and Weeks (3) found that, by reflex action, warming of the vascularly isolated glomus increased respiration and vasoconstriction, whereas cooling had the opposite effect. They give the tentative explanation that according to the law of van't Hoff increased acidity within the glomus cells will occur more rapidly as temperature and metabolism in the organ rise. Euler, Liljestrand and Zotterman (11) observed that a stepwise reduction of the number of functioning chemoreceptive fibres in the sinus nerve reduced the frequency of potentials in response to both oxygen want and carbon dioxide in the same proportion. Intravenous injection of a small dose of ammonia, and the same holds true for sodium carbonate, led to a sudden temporary disappearance of the action potentials set up by oxygen want or carbon dioxide. These observations suggest strongly that both kinds of stimuli act by increasing the cH in the chemoreceptors.

Though there can be no doubt about the importance of the intracellular acidity of the carotid body for its response to physiological stimuli, other observations clearly demonstrate that this cannot be the only determining factor. Thus a number of drugs which act on autonomic ganglia, such as nicotine, lobeline and acetylcholine, also exercise a strong stimulation on the chemoreceptors of the carotid body. There seems to be little reason to assume that the very small doses of these drugs which suffice to elicit this action should have an appreciable influence on the intracellular acidity. Hence some other mode of action must be sought. In 1938 Schweitzer and Wright (29) emphasized the possibility that drugs, or changes in the blood which stimulate the chemoreceptive nerve endings, produce their effects by liberation of acetylcholine as the chemical intermediary. Since the enzymatic splitting of this substance proceeds at a slower rate with increased acidity, within physiological limits, one must expect an accumulation of acetylcholine under these circumstances and an enhanced response of the chemoreceptors in conformity with the theory of the acid-neurohormonal

mechanism of gradation of nerve cell activity as suggested by Gesell, Brassfield and Hamilton (14).

Heymans, Bouckaert and Pannier (17), as well as Fernandez (13), have raised objections against the theory that acetylcholine is a transmitter substance in the carotid body, but their experimental results are by no means convincing. The question was taken up again by Liljestrand (26), who found application of 0.1 to 0.2 per cent of eserine salicylate to both carotid bodies to cause an increase in ventilation. On the other hand, one per cent atropine sulphate under similar conditions decreased or abolished the response of the chemoreceptors to low oxygen pressure as well as to lobeline.

These results led to a detailed study of the effect of a number of autonomic drugs on the chemoreceptor action potentials of the sinus nerve by Landgren, Liljestrand and Zotterman (21). As a rule the substances tested were dissolved in 1 ml. Ringer solution and injected into the thyroid artery. The blood flow through the carotid body according to de Burgh Daly, Lambertsen and Schweitzer (8) is about 0.06 ml. per minute, and similar values were obtained by Landgren, et al (21). With the method used, only about $\frac{1}{200}$ of the injected amount will reach the carotid body, and still less will penetrate into the cells. Sometimes the contact between the injected fluid and the carotid body was prolonged by clamping the common carotid arteries centrally to the place of injection. A number of drugs which inhibit cholinesterases enhanced more or less the chemoceptor activity in the sinus nerve during oxygen- or air-breathing as well as during oxygen want. Such results were obtained with eserine, prostigmine, DFP, TEPP, ergotamine, morphine and sodium fluoride, and—in later experiments—also with a number of aliphatic alcohols, acetone, ether, chloroform, and some purine derivatives (22, 23, 27). Greatly increased activity was observed during oxygen inhalation after 250 microgm. of prostigmine, and a corresponding result after 1 mgm. of TEPP. In the latter case the effect remained for a long time, as would be expected with a substance that combines irreversibly with cholinesterases. Since the inhalation of 5.6 per cent oxygen in nitrogen did not further increase the potentials, it seems probable that the response was already maximal. This would also explain why close arterial injection of 1 ml. of 0.05 N ammonia, which temporarily abolishes or strongly reduces the stimulating effects of citrate, fluoride or lobeline on action potentials, had practically no effect. Since the cholinesterases were completely inactivated, no splitting of acetylcholine took place even if the intracellular reaction was displaced towards the alkaline side.

Acetylcholine antagonists such as atropine, curarine, tetraethylammonium, hexamethonium and decamethonium decreased or abolished the response elicited by lobeline, acetylcholine or oxygen want, the effect being considerably more pronounced against the alkaloids.

A strong stimulating effect was observed after succinylcholine. This is of special interest since Granit, Skoglund and Thesleff (15) have recently described a great increase in the electrical activity of the afferent nerves from the muscle spindles after intravenous injection of 0.1 to 0.2 mgm. of this substance.

A paralyzing effect of monoiodoacetate on the chemoreceptors of the carotid

body was described by Winder (31) and by Anitschkov (2). The intracarotid injection of 10 mgm. monoiodoacetate in our experiment quickly led to abolition of all chemoreceptor impulses; even 10 microgm. of lobeline sulphate, which had a strong stimulating effect immediately before the application of the lobeline, now failed to induce any activity. But it can also be seen that the previous small dose of 2 mgm. monoiodoacetate had a pronounced stimulating effect—in fact, more than did 6 per cent oxygen. This would hardly be expected from the blocking effect of lactic acid formation, but can easily be understood if one keeps in mind that monoiodoacetate also inhibits cholinesterases as well as choline acetylase. Since the former effect will appear at first, this will give a satisfactory explanation. According to Nachmansohn (28) monoiodoacetate interferes not only with carbohydrate metabolism but also with the "acetylcholine cycle", so that the results seem to be in accordance with the theory that acetylcholine acts as a chemical transmitter.

Probably the influence of temperature observed by Bernthal and Weeks (3) may also be reconciled with this theory. Even if cell acidity during oxygen want rises more rapidly if the temperature of the carotid body is elevated, there is also a more rapid enzymatic breakdown of acetylcholine at a definite pH, as Abdon and Uvnäs (1) have shown. How the synthetic action of choline acetylase would be affected is unknown. There are obviously a number of chemical reactions involved in the breakdown and resynthesis of acetylcholine, and experiments evaluating the net result of changes in temperature are necessary.

According to the determinations of Hollinshead and Sawyer (18) the carotid body contains unspecific as well as specific cholinesterases, the former being present in larger amounts than the latter. Using histo-chemical methods Koelle (19, 20) has arrived at the same conclusion. He points out that the presence not only of the specific but also of the unspecific enzyme indicates some important function. It must be admitted that as yet a release of acetylcholine from the active carotid body has not been definitely proved, and the possibility cannot be excluded that the transmitter substance consists of some unknown choline ester with a general behavior and action similar to those of acetylcholine. This would harmonize well with the relatively large amounts of unspecific cholinesterases. In this connection it is of interest to again draw attention to the pronounced stimulating effect of succinylcholine.

Acetylcholine is not the only substance that has been considered as a chemical transmitter in the carotid body. According to Fabinyi and Szebehelyi (12), increase in respiration after inhalation of 10 per cent oxygen can be prevented by cholesterol, by antistine, or by desensitization to histamine. These observations suggested that histamine might be concerned with the stimulation of the chemoreceptors at low oxygen pressure. However, this could not be confirmed by Å Liljestrand (25). Intravenous injection of the antihistaminic substance lergitin (N-phenyl-N-benzyl-N′N′ dimethylethylenediamine hydrochloride), in doses that suppressed the blood pressure action of moderate amounts of histamine in the cat, did not diminish the response to oxygen lack. In accordance with this observation 2 to 50 microgm. of histamine hydrochloride given by intracarotid in-

jection did not induce any increased activity in the sinus nerve during oxygen breathing (23). Nor did 1 mgm. lergitin diminish the potentials voked by 6 per cent oxygen. If the dose was raised to 10 mgm. the response to oxygen lack nearly disappeared but this was probably due to the local anesthetic action of the drug.

The results obtained with the carotid body made it desirable that similar experiments should be performed with other sets of chemoreceptors. Landgren, Zotterman and Liljestrand (22) have recently tried the effect of a number of the substances already mentioned on taste receptors in the tongue of the frog (*Rana esculenta*). The action potentials from the lingual branch of the glossopharyngeal nerve were recorded as an indicator of the activity. Two kinds of physiological stimuli were used and standardized so that uniform conditions prevailed. One, the water test, depends on the observation by Zotterman (32) that tap water applied to the frog's tongue elicits a massive volley of large fibre impulses in the nerve, initially set off both by deformation of the tongue and stimulation of the "water taste" receptors and later, after the former effect has vanished, by the effect on the taste organs alone. The second test consisted of the application to the tongue of a small pad of cotton soaked with a solution of 1.3 or 2 per cent sodium chloride. The impulses were recorded 15 seconds after the application of the salt solution. Control experiments were perfórmed with frog Ringer. The different substances to be investigated were applied in the same way as the salt solution.

The effect of a weak solution of acetylcholine (1:10,000) was as follows. After a few minutes spontaneous impulses were reduced, and responses to the application of 1.3 per cent sodium chloride or to the water test had disappeared. The paralysis was reversible, however, and washing with Ringer soon restored the excitability. In fact, it was easy to find a point when the water test called forth a much stronger response than during the control period. A similar enhancement of the sensitivity for sodium chloride was observed. Depending upon the concentration, acetylcholine thus can produce a state of increased sensitivity or of paralysis of the taste buds.

With prostigmine (2.5:10,000) an increased spontaneous activity occurred, and the responses to both tests were greatly enhanced. Very similar results were obtained with DFP (1:1,000) and TEPP (1:1,000), whereas after d-tubocurarine (3:1,000) and decamethonium (1:1,000 to 1:500) a considerable reduction—without primary sensitization—to both tests was obtained. Succinylcholine (1:10,000) gave an enormous increase of spontaneous impulses and a moderate increase after water or sodium chloride, but soon paralysis became obvious.

There is undoubtedly a close analogy between the reactions to a number of drugs of the chemoreceptors of the frog's tongue and those of the carotid body of the cat. In both cases an enhanced activity could be obtained by acetylcholine as well as by drugs that inhibit the cholinesterases. Larger amounts of these drugs led to paralysis of both kinds of receptors. *d*-Tubocurarine only inhibited the receptors as did decamethonium. However, the latter drug first produced a discharge of large spikes before a diminution of the response to the physiological stimuli could be observed. The extraordinarily strong stimulating action of succinylcholine occurred with both preparations.

These observations strongly favor the view that acetylcholine—or some closely related substance—has a similar function for the initiation of impulses in taste receptors as well as in the carotid body. It is interesting to note that Brücke, Hellauer and Umrath (7) have found the acetylcholine content to be about 3 times greater in the foliate papillae of the rabbit than in other parts of the mucous membrane of the tongue. They also observed a considerable lowering of the content in the papillae, but not in the adjoining mucous membrane, 6 to 24 days after severing of the glossopharyngeal nerve. These results are in good agreement with the view expressed here.

Nothing seems to be known concerning the occurrence of some chemical transmitter substance in the smell receptors. It would probably not be difficult to ascertain whether a situation exists corresponding to that of taste receptors.

In the study on the frog's tongue referred to, the abolition of action potentials caused by touch was regularly observed following the paralysis of the chemoreceptors, and there also were indications of increased excitability to touch simultaneously with such an effect on the chemoreceptors. Thus, in addition, acetylcholine would seem to be concerned with the origin of impulses after stimulation of touch receptors. In this connection it seems appropriate to call attention to recent work on the effect of acetylcholine and certain autonomic drugs on some sensory nerve endings. Brown and Gray (6) after injection of small doses of nicotine or acetylcholine into the skin or mesentery of cats and dogs found a centripetal discharge of impulses in the nerves supplying the injected area. Bing and Skouby (4) observed that the introduction of small amounts of cholinergic substances into the skin produced an increased number of cold spots, and Skouby (30) noted that acetylcholine injected into the skin lowered the pain threshold. Dodt, Skouby and Zotterman (10) stated that acetylcholine injected locally shifted the temperature range of the thermal receptors of the tongue of the cat towards the warm side.

It seems, therefore, that acetylcholine or some similar substance plays an important role as a transmitter for a number of receptors. The exact nature of this transmitter for the different receptors and its mode of action will be an object for future research.

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