EFFECTS OF CO_2 AND HYDROGEN IONS ON THE PHYSICAL ELECTROTONUS OF SMOOTH MUSCLE

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The investigations of the author have already shown that the exposure of smooth muscle to a gaseous mixture of 5% CO_2 and 95% O_2 is accompanied by reduction of physical electrotonus, depression of spontaneous activity and reduction of excitability in the muscle. The observation of these changes in smooth muscle led to a more detailed examination of the effect of CO_2 on physical electrotonus. The author has been unable to find any published information on this subject. At the same time, similar investigations on other excitable tissues indicate that CO_2 may produce a variety of effects on the electrical properties of such tissues [6, 10-12].

METHOD

The material on which the investigation was carried out was the annular smooth muscle of frog stomach. The methods employed for examination of physical electrotonus and for production of the required gaseous mixture in a sealed moist chamber have already been described [3, 4]. The gaseous mixture was $^2/_3$ - $^3/_4$ O_2 or N_2 and $^1/_3$ - $^1/_4$ CO_2. Nitrogen (which, however, contained 2-3% O_2) was used in some experiments in place of O_2. The H ion concentration of Ringer's solution was reduced to 2.0-2.5 by replacing $NaHCO_3$ with an equivalent quantity of HCl. A solution of the required pH value (which was measured electrometrically) could then be obtained by adding a suitable quantity of the acid Ringer's solution to the normal solution with a pH of 7.0.

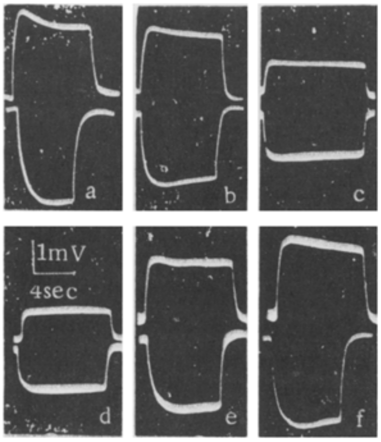

Fig. 1. Change in physical electrotonus of muscle resulting from action of 2 : 1 O_2/CO_2 gaseous mixture. a) In normal state; b, c, d) after action of gaseous mixture for 5, 15, and 30 min; e, f) 2 and 5 min after withdrawal of gaseous mixture and removal of cover from moist chamber. Upper tracing - catelectrotonus. Lower tracing - anelectrotonus.

RESULTS

Exposure of smooth muscle to the action of the gaseous mixture was accompanied by suppression of spontaneous activity, suppression of muscle excitability and considerable reduction of physical electrotonus. With an exposure of 15-25 min, physical electrotonus was reduced by 50-70%, catelectrotonic potential being reduced much more than anelectrotonic. Continued exposure did not lead to any significant further reduction of electrotonus. When the gaseous mixture was withdrawn and the cover removed from the chamber, physical electrotonus regained its initial value absolutely in the course of a few minutes.

Figure 1 is from an experiment in which the muscle was exposed to a gaseous mixture of O_2 and CO_2 in proportions of 2 : 1. The strength of the polarizing current was 9 μA. Before the gaseous mixture was brought into contact with the muscle, a fairly considerable negative potential developed towards the end of the ascending part of

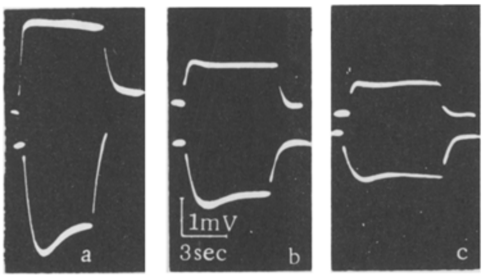

Fig. 2. Changes in anelectrotonus produced by a 4:1 O_2/CO_2 mixture. a) In normal state; b,c) after action of gaseous mixture for 10 and 20 min. Tracings as in Fig. 1.

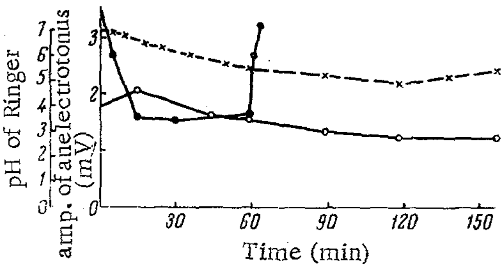

Fig. 3. Changes in amplitude of anelectrotonic potential (black circles) and pH of Ringer's solution (crosses) and changes in amplitude of anelectrotonus (white circles) produced by acidified Ringer's solution (pH 5.0).

of the catelectrotonic potential, after which the tracing became established at a relatively constant level (Fig. 1a). The incremental phase of the catelectrotonic potential lasted 0.33 sec. After the gaseous mixture had been passing through the chamber, there was appreciable reduction of both the negative potential and the amplitude of catelectrotonic potential (Fig. 1b). After 15 min amplitude was down to less than half, and no negative potential developed (Fig. 1c). The incremental phase of catelectrotonic potential was then reduced to 0.085 sec. There was little or no further change in catelectrotonic potential as a result of the continued action of the gaseous mixture (Fig. 1d). Catelectrotonic potential regained its original value and negative potential again developed at the end of the incremental phase within a few minutes after withdrawal of the CO_2 mixture and removal of the cover of the chamber (Fig. 1e, f). Figure 1 also shows that the amplitude of anelectrotonic potential (deflection downwards) was also considerably reduced by the action of the gaseous mixture (Fig. 1a-d). The incremental time for anelectrotonic potential suffered reduction from 0.5 to 0.3 sec. Just as in the normal state, however, the difference between the values of catelectrotonic and anelectrotonic potential was not abolished by the action of the gaseous mixture, and the amplitude of anelectrotonus remained somewhat greater than the amplitude of catelectrotonus.

Figure 2 is from an experiment in which muscle was exposed to a 4:1 mixture of O_2 and CO_2. The strength of the polarizing current used was purposely greater than the strength required for tracing change in the "rise" of anelectrotonus.

Under normal conditions, the amplitude of anelectrotonus was considerably greater than that of catelectrotonus and, at the end of its ascending part, there was a quite considerable "jump," after which the potential settled at a constant level (Fig. 2a). The amplitude of anelectrotonus potential was considerably reduced after 10 min exposure to the CO_2 mixture, and the "jump" was even more markedly reduced than the amplitude (Fig. 2b). When the polarizing current was withdrawn, negative potential no longer developed at the end of the descending part of the anelectrotonic tracing. All these changes in anelectrotonus were still more pronounced after exposure to the gaseous mixture for 20 min (Fig. 2c).

Considerable reduction of physical electrotonus was also observed when O_2 in the mixture was replaced by N_2. This was evidence that the reduction of physical electrotonus, seen under these conditions, was due to the effect of CO_2 on the muscle, and not effects produced by the other gases.

It is, however, still uncertain whether CO_2 has any direct specific action on muscle or whether its effect is produced indirectly, perhaps through CO_2-determined change in H ion concentration.

The pH value of Ringer's solution, placed in a sealed moist chamber, through which a 2:1 O_2/CO_2 mixture was passed, was therefore determined. The effect of the acidified form of Ringer's solution on the physical electrotonus of smooth muscle was also studied (Fig. 3).

The pH of the Ringer's solution fell gradually to 5.0 in the course of exposure to the gaseous mixture for 2 h. When, after 2 h, the gaseous mixture was withdrawn and the cover of the chamber removed, the pH value rose to 6.0.

In contrast to these changes, the amplitude of anelectrotonus was reduced to about half its initial value by exposure of the muscle to the gaseous mixture for 15 min. Its amplitude reached its minimum value after 30 min and then began to increase slightly. Withdrawal of the mixture after 60 min resulted in rapid restoration of amplitude. There was thus no direct relationship between increase of acidity of the Ringer's solution and reduction of anelectrotonus amplitude produced by action of the same gaseous mixture.

Figure 3 also shows that the amplitude of the anelectrotonic potential increased slightly when the muscle was first exposed to the action of the acid form of Ringer's solution (pH 5.0), and then declined very gradually. Catelectrotonic potential underwent similar changes.

The reduction of physical electrotonus by acid Ringer's solution (pH 5.0) thus failed to coincide, either in time or in magnitude, with the reduction produced by action of the gaseous mixture. Even with still more acid Ringer's solution (pH 3.0) reduction of physical electrotonus, though still considerable, proceeded more slowly than with the gaseous mixture. Furthermore, subsequent washing of the muscle with normal Ringer's solution failed to restore the value of physical electrotonus, even when continued for $1^1/_2$ h.

All these results indicate that the changes in physical electrotonus, observed to result from exposure to the gaseous mixture, were connected with the direct specific action of CO_2 on the muscle, and not with the action of hydrogen ions. It is suggested that the reduction of physical electrotonus under the conditions described was due to increased permeability of the membranes of the smooth muscle cells for ions as, other things being equal, increase of physical electrotonus depends on the state of membrane permeability. This may also be the explanation for the reduction of potassium ion concentration produced in striated muscle by the action of CO_2 [5].

The specific and very rapid attenuating effect of CO_2 on physical electrotonus is probably connected with its action on certain excessively sensitive metabolic processes controlling the permeability of the cytoplasmic membrane. Root [14] has shown, for example, that CO_2 inhibits oxidative metabolism in nerve. Meves and Völkner [11] found that depolarized muscle fibers, exposed to CO_2, became repolarized or even hyperpolarized. If, however, the muscle fibers exposed to CO_2 had been depolarized with dinitrophenol, repolarization did not occur and, in fact, there was further decline of resting potential.

The changes in electrotonus produced by acid Ringer's solution were less marked. The resting membrane potentials of striated muscle fibers also showed little change when the acidity of Ringer's solution was increased to 5.0 and 4.0 [9-11, 13]. The initial slight increase of electrotonus in smooth muscle was probably connected with the action of calcium ions, as calcium dissociation is known to increase in acid solution. The subsequent reduction of electrotonus was apparently due solely to increase of membrane permeability produced by hydrogen ions.

Increase of calcium ion concentration in Ringer's solution was, in fact, associated with increase of electrotonus in these experiments. Heene [8] has also observed this calcium effect in nerve in acidified Ringer's solution (pH 5.0).

SUMMARY

Under the influence of a carbonate gas mixture ($^2/_3 - ^3/_4\ O_2 + ^1/_3 - ^1/_4\ CO_2$) the physical electrotonus (PE) of the circular smooth muscles of the frog stomach very quickly decreases 50–70%, while the excitability and spontaneous activity of the muscle are completely inhibited. These changes are quickly and easily reversible and are not connected with the action on the muscle of H^+ forming under the influence of CO_2 inasmuch as in a deliberately acidic Ringer's solution with pH reduced to a level close to that seen after the passage of a carbonate gas mixture, the PE at first slightly increases and then very slowly and only insignificantly lessens. Changes in the electrical properties of the smooth muscle, and, in particular, the decline of the PE under the influence of the carbonate gas mixture are apparently associated with the direct specific action of CO_2 on the permeability of the protoplasmatic membrane.

LITERATURE CITED

1. P. G. Kostyuk and Z. A. Sorokina, In: Membrane Transport and Metabolism. Prague (1961), p. 193.
2. Z. A. Sorokina, Citologiya, No. 1 (1961), p. 48.
3. M. F. Shuba, Fiziol. Zh. SSSR, No. 8 (1961), p. 1068.
4. M. F. Shuba, Fiziol. Zh., No. 2 (1964), p. 156.
5. M. L. Audibert, F. Bermond, and F. Filippi, J. Physiol. (Paris), Vol. 49 (1957), p. 28.
6. J. Boistel, E. Coraboeuf, and J. Guérin, J. Physiol. (Paris), Vol. 49 (1957), p. 53.

7. P. C. Caldwell, J. Physiol. (London), Vol. 126 (1954), p. 169; Ibid. Vol. 142 (1958), p. 22.
8. R. Heene, Pflüg. Arch. Ges. Physiol., Vol. 274 (1961), p. 27.
9. H. P. Jenerick and R. W. Gerard, J. Cell. Comp. Physiol., Vol. 42 (1953), p. 79.
10. G. Ling and R. W. Gerard, J. Cell. Comp. Physiol. Vol. 34 (1949), p. 383.
11. H. Meves and K. G. Völkner, Pflüg. Arch. Ges. Physiol., Vol. 265 (1958), p. 457.
12. A. M. Monnier, Ergebn. Physiol., Vol. 48 (1955), p. 230.
13. P. Pauschinger and W. Kutscha, Pflüg. Arch. Ges. Physiol., Vol. 274 (1961), p. 4.
14. W. S. Root, J. Cell. Comp. Physiol., Vol. 1 (1932), p. 20.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. ***Some or all of this periodical literature may well be available in English translation.*** **A complete list of the cover-to-cover English translations appears at the back of this issue.**