Effect of Procaine, Calcium, Magnesium and Potassium on the Acetylcholine Release from the Sweat Nerve Endings in Human Skin

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The effects of procaine, Ca, Mg and K on the release of transmitter from the cholinergic sweat nerve endings in the forearm skin were studied. Sweating was visualized by the iodine-starch method of Wada and Takagaki.

At a concentration of 10^{-7} , intradermal injections of eserine did not produce a local sweating in a cool environment, but they became effective in eliciting sweat response on the injection wheals by entering a warm or hot environment. Local sweat responses to eserine in 10^{-6} and 10^{-5} were also intensified on exposure to heat.

Procaine in 10^{-4} inhibited the sweat responses to eserine very probably by affecting the acetylcholine release from the sweat nerve endings. It was found that Ca ions contained in Krebs solution are important in the process of the acetylcholine release and excessive Ca facilitates this release. In contrast, Mg and K ions present in Krebs solution were without any effect. But, when Mg was in excess relative to Ca, it was observed that Mg antagonized the effect of Ca. The antagonism between Mg and Ca was demonstrated also in the case of spontaneous thermoregulatory sweating. The effect of Ca on the acetylcholine release seems to be antagonized by Mg in a ratio of approximately 1:2 in molality.

In 1933 Dale and Feldberg^{1,2} demonstrated the release of acetylcholine from the sympathetic nerves supplying the sweat glands of the cat's foot-pads, and deduced therefrom that the sweat glands of human skin are likewise innervated by cholinergic sympathetic nerve fibers. Since then, this has been generally believed to be true, although a direct experimental evidence is still lacking concerning the acetylcholine release from human sweat nerve fibers. Nevertheless, our knowledge regarding local factors which may influence this transmitter release in human skin is still scanty.

The present investigation was attempted to study the effects of procaine, calcium, magnesium and potassium upon the release of acetylcholine from the sweat nerve endings, when local sweat responses were elicited by intradermal injections of eserine in a warm or hot environment and when spontaneous thermoregulatory sweating was produced in a hot environment.

METHODS

In a total of 12 male subjects consisting of the present authors and medical students, usually the dorsal surface and sometimes the ventral surface of the forearms were used as test areas.

In the first series of experiments, local sweating was produced by intradermal injection of eserine in a warm environment, and in the second series, spontaneous thermoregulatory sweating was induced over the test areas by entering a hot environment.

Sweating was visualized by the iodine-starch method of Wada and Takagaki.^{3,4} Three or five per cent iodine in absolute alcohol was painted over the test areas, and when dried completely, a water-free suspension of corn-starch and castor oil (1:1 in volume) was painted over the iodine-treated areas. Sweat at each active sweat duct opening was visualized as a black spot, as illustrated in the figures attached to the present paper. Observations of sweating were made routinely under a magnifying lens. This method was proved to have an additional advantage in that the black spots can be removed by wiping with cotton-wool pledget soaked with absolute alcohol, and then the indicator materials for visualizing sweat can be reapplied; and thus the change in the density of sweat spots on the test areas and the duration of sweating can be determined.

In the first series of experiments the effects of procaine, Ca, Mg, and K upon the release of acetylcholine from the sweat nerve endings were studied, preventing destruction of acetylcholine by intradermal injections of eserine in 10^{-7} , 10^{-6} , 5×10^{-6} and 10^{-5} . Eserine (*Physostigminum puriss.*, Merck) was dissolved in glucose-free Krebs solution containing 0.692% NaCl, 0.0354% KCl, 0.0282% CaCl_2 , 0.210% NaHCO_3 , 0.0162% KH_2PO_4 and 0.0294% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; or in 0.9% NaCl solution, unless otherwise stated. Krebs solution or 0.9% NaCl solution or both were used as controls.

In this series of experiments procaine hydrochloride, CaCl_2 , MgCl_2 and KCl were dissolved in Krebs or 0.9% NaCl solution containing eserine in concentrations effective in eliciting sweat response in warm environment, eserine-containing Krebs and/or eserine-containing 0.9% NaCl solution being used as control.

A test solution or solutions and a control solution or solutions were injected intradermally in an amount of 0.2 ml for each solution, and injection wheals, 2 to 4 in number, were made along a long axis of the forearm at an interval of 3 to 4 cm between the centers of two neighboring wheals. Usually, the injections were made in a cool room at 2.5 to 23.0°C with relative humidity of about 35 to 90%. Elapsed time after the start of injection was measured for each injection wheal with stop-watches. In the majority of experiments sweat responses to injected eserine were induced by entering usually a warm or sometimes hot room at 28.5 to 39.0°C with relative humidity of about 20 to 85%, within 3 to 5 min after the end of the last injection. Observations of sweat response to eserine could be continued until spontaneous thermoregulatory sweating began to appear over the entire test areas including the injection wheals. Therefore, the environmental temperature must be kept at an appropriate level for the purpose of prolonging the observation period.

In the second series of experiments, where the effects of Mg and Ca ions on the thermoregulatory sweating were studied, test solutions were prepared by adding MgCl_2 or by adding MgCl_2 and CaCl_2 to Krebs solution. Two to four injection wheals consisting of a test solution or solutions and a control solution or solutions, were made in such a way as described above in a cool environment, and 1.5 to 3 min after the end of the last injection the subject was led to enter a hot room at 35 to 40°C with relative humidity of about 50 to 70%. Sometimes, the injections were performed in a hot environment. The effect of test solutions on the thermoregulatory sweating was evaluated by comparing the time of appearance and density of sweat spots on the wheals produced by injections of test solutions with those on the control wheals. In most of experiments, the observations were continued until the density of sweat spots on the injection wheals produced with test and control solutions became almost identical.

RESULTS

1) The relation of sudorific effect of intradermal injection of eserine to environmental temperature

In preliminary experiments the effects of eserine dissolved in Krebs solution at concentrations of 10^{-7} , 10^{-6} and 10^{-5} were examined at room temperature of 3 to 12°C. In 5 of 6 tests with eserine in 10^{-7} no sweat response was obtained during the observation period of 20 min; in the remaining one it began to appear at 11 min after injection. With eserine in 10^{-6} , positive results were gained in 6 of 7 tests; the latent period before the appearance of sweating on the injection wheal ranged from about 5 to 19 min. Further, with eserine in 10^{-5} a more definite sweat response was found to occur in all 7 tests on 4 subjects; sweat spots began to appear about 1 min and 30 sec to 4 min in 6 out of the 7 tests and about 9 min in the remaining one test. Although the tests were small in number, it was noticed that the responses to effective concentrations of eserine were variable in the duration of latency for sweating and in its intensity at different times even in one and the same individuals.

Within 4 to 5 min after the start of injection of eserine in 10^{-9} to 10^{-5} in a room at 8 to 18.5°C, the test subject entered another room at 23 to 34.5°C. It was of interest to find that soon after the subject had felt hotness a more or less marked sweating began to appear always on the wheal produced by injection of eserine in 10^{-7} and higher concentrations (cf. Fig. 1). Eserine in 10^{-7} was effective in all 10 tests; but eserine in 10^{-8} also showed positive results in 6 of 9 tests. No response was obtained with eserine in 10^{-9} . The appearance of the response to effective concentrations of eserine was promoted by drinking hot water or by application of radiant heat to any part of the body other than the test areas. It should be noted that the effect of eserine must be assessed before spontaneous thermoregulatory sweating began to appear over the test areas.

Usually, the sweat response to eserine in 10^{-7} could be interrupted when the subject returned to a cold or cool environment. By repeatedly changing the environment, it was found that the effect of eserine in 10^{-7} persisted for at least 30 min, and that of eserine in 10^{-5} for about 1.5 to 2 hours after injection. In general, the response to eserine in 10^{-7} was restricted mainly to the injection wheal, and the response to eserine in 10^{-6} and 10^{-5} tended to extend to the areas surrounding the injection wheal, as time passed (Fig. 1).

These findings provide indirect but good evidence that the transmitter of the sweat nerve fibers is acetylcholine. In the present experiments a concentration of eserine as low as 10^{-7} was found to be sufficient to induce sweating by its anticholinesterase action, even when the amount of acetylcholine released on exposure to a warm or hot environment was still subthreshold for stimulating the sweat glands.

It should be noted here that the latent period between the injection and the appearance of response on the injection wheals, especially produced with relatively

Fig. 1. Sweat responses to eserine. Three wheals were produced, from left to right, by intradermal injections of 0.2 ml of Krebs solutions containing eserine in 10^{-5} , 10^{-6} and 10^{-7} . The injections were made at intervals of 29 and 35 sec respectively between the two successive injections, at 11°C dry bulb and 9.5°C wet bulb.

Upper photograph was taken at 5 min, and lower one was taken at 15 min after the start of injection of eserine in 10^{-5} .

Sweat spots began to appear only on the wheal with eserine in 10^{-5} at about 2 min after the start of its injection. About 4 min later, the subject entered a hot room at 38°C dry bulb and 24°C wet bulb, and took a cup of hot water. About 2 min after this, sweat spots became visible also on the wheals with eserine in 10^{-6} and 10^{-7} . The extent of the response increased with increase in the concentration of eserine. Note: the response to eserine in 10^{-7} is restricted to almost the entire surface of its injection wheal. $\times 1.5$.

low concentrations of eserine was variable, even when the same concentrations of eserine were repeatedly tested on one and the same subject. This can be explained by assuming that the onset of response depends upon the internal and external conditions necessary for the excitation of the sweat nerves; that is, it depends upon the amount of acetylcholine released from their endings.

2) *Effect of procaine on the sudorific effect of eserine*

A total of 16 experiments were carried out to determine the effect of procaine on the sweat responses to intradermal eserine in 10^{-7} , 10^{-6} and 10^{-5} . Procaine was given in mixture with eserine in Krebs solution.

It was found that procaine in 10^{-4} usually delayed at least for about 20 min the appearance of sweating produced by eserine in 10^{-7} , as compared with that with control injection of eserine in 10^{-7} alone. At this concentration of procaine, the density of sweat spots was evidently low, even when they appeared evenly on the injection wheal with the mixture of procaine and eserine. Likewise, procaine in 10^{-4} inhibited the response to eserine in 10^{-6} .

It should be noted here that this inhibitory action of procaine might not be localized to the sweat glands themselves, since local sweating produced by intradermal injection of acetylcholine in 10^{-7} was never affected by procaine in 10^{-4} in all 4 tests.

The inhibition by procaine was more marked or complete at a concentration of 5×10^{-4} not only on the sweat response to eserine in 10^{-7} but also on that to eserine in 10^{-6} and 10^{-5} (Fig. 2). With procaine in 10^{-3} , the response to eserine in 10^{-5} was almost completely inhibited not rarely for about one hour after injection; sweat spots appeared, if any, in the areas surrounding the wheal. But, when spontaneous thermoregulatory sweating appeared over the test areas, it was hardly inhibited on the wheal with procaine in 10^{-4} , whereas it was markedly inhibited on the wheal with procaine in 10^{-3} .

From these results it seems reasonable to assume that the release of acetylcholine which is below the threshold for provoking sweat response may be blocked by procaine in a concentration as low as 10^{-4} .

3) *Effect of calcium ions on the sweating by eserine*

Effect of eserine in Ca-free medium. In 12 experiments the effects of eserine in 10^{-7} , 10^{-6} and 10^{-5} dissolved in Ca-free Krebs solution were studied. In all experiments the sweat responses to these concentrations of eserine were definitely inhibited by the removal of Ca ions. The inhibition was most marked with eserine in 10^{-7} in the Ca-free medium. For example, sweat spots began to appear about five and a half minutes after injection on the wheal with 10^{-7} eserine in Krebs solution and gradually increased in number to reach over 20 spots at the end of 30 minutes, while no response occurred on the wheal with Ca-free Krebs containing the same concentration of eserine. Likewise, the responses to eserine in 10^{-6} and 10^{-5} were also affected by removal of Ca ions: the appearance of response was delayed for

Fig. 2. Inhibitory effect of procaine on the sweat response to eserine. Four wheals were produced, from left to right, by intradermal injections of 0.2 ml of 1) Krebs solution containing eserine in 10^{-5} ; 2) Krebs solution containing eserine in 10^{-5} plus procaine in 5×10^{-4} ; 3) Krebs solution containing eserine in 10^{-6} ; and 4) Krebs solution containing eserine in 10^{-6} plus procaine 5×10^{-4} . The injections were made at intervals of 30, 24 and 27 sec respectively between the two successive injections, at 22°C dry bulb and 17°C wet bulb.

Only on the wheal with eserine in 10^{-5} , sweat spots began to appear at 2 min and 18 sec after the start of its injection.

At 6 min after the start of injection of the first solution, the subject entered a hot room at 36°C dry bulb and 22.5°C wet bulb. About 30 sec later, sweat spots began to appear also on the wheal with eserine in 10^{-6} . Photographed at 11 min after the start of injection of the first solution. Note: there is no sweat response on the wheals with the procaine added solutions. $\times 1.2$.

about 1 to 4 min, and the number and size of sweat spots were small for 15 to 35 min after injection, as compared with the response on the control wheal.

Similar results were obtained with eserine dissolved in 0.9% NaCl. In all 9 experiments it was found that the sudorific effects of eserine dissolved in 0.9% NaCl at 5×10^{-7} , 10^{-6} and 5×10^{-6} were clearly weaker than those of identical concentrations of eserine in Krebs solution: the appearance of the sudorific effect of eserine in 0.9% NaCl was delayed for 3 to 5 min or more, and the density of sweat spots was markedly less, as compared with the response to identical concentrations of eserine in Krebs.

These results suggest that Ca ions in Krebs solution (2.5 mM Ca) play an essential role in the acetylcholine release from the sweat nerve endings under the present experimental conditions.

Effect of eserine in Ca-containing medium. The importance of Ca ions in the sudorific effect of eserine was confirmed by further experiments in which a com-

Fig. 3. Effect of Ca on the sweat response to eserine. Three wheals were produced, from left to right, by intradermal injections of 0.2 ml of 1) Krebs solution containing eserine in 10^{-6} ; 2) 0.9% NaCl solution containing eserine in 10^{-6} plus Ca in 2.7 mM; and 3) 0.9% NaCl solution containing eserine in 10^{-6} . The injections were made at intervals of 25 and 30 sec respectively between the two successive injections, at 17.5°C dry bulb and 13°C wet bulb.

At 3 min after the start of injection of the first solution, the subject entered a hot room at 32°C dry bulb and 20.5°C wet bulb. At about 4 min after the start of injection of the first solution, sweat spots began to appear almost simultaneously on the wheals with the eserine-Krebs solution and with the Ca added eserine-NaCl solution. On the wheal with the eserine-NaCl solution, sweat spots became visible at 6 min and 30 sec after the start of its injection.

Photographed at 9 min after the start of injection of the first solution. Note: sweat spots on the wheal with the eserine-NaCl solution are small in number and size, as compared with those on two wheals with the Ca containing solutions. $\times 1.5$.

parison was made between the effect of eserine in Krebs solution and the effect of eserine in 0.9% NaCl solution to which CaCl_2 was added at a concentration of 4×10^{-4} (2.7 mM Ca); there was no significant difference in both the appearance and magnitude of sweat response between the injection wheals produced with these two solutions in all 15 experiments (Fig. 3).

In a total of 14 experiments, the effects of eserine at 10^{-6} and 5×10^{-6} in 0.9% NaCl solution to which CaCl_2 was added at 10^{-3} (9 mM Ca) or at 5×10^{-3} (45 mM Ca) were examined. Control solutions were made by adding osmotically equivalent amounts of NaCl, in place of CaCl_2 , to Krebs and 0.9 % NaCl solutions containing each concentration of eserine. In all of 7 experiments with CaCl_2 in 10^{-3} and of another 7 experiments with CaCl_2 in 5×10^{-3} , the sudorific effect of eserine was influenced by its solvents: it diminished in the order of the NaCl solution containing CaCl_2 , Krebs solution, and the NaCl solution containing no CaCl_2 . The latent period before the appearance of response was usually shortened and the size of sweat spots was larger on the wheal produced with the NaCl solution to which CaCl_2 was added at relatively high concentrations, as compared with those produced with

the other two solutions. This was confirmed by repeated application of the indicator materials for visualizing sweat to the test areas, suggesting that the secretion rate of sweat was increased in the presence of excessive Ca. Not infrequently, it was observed that even when the response to the control eserine-containing solutions had ceased, the response to eserine-containing Ca-rich solutions was found to have still persisted. Similarly, in additional four experiments the effect of Ca ions in 5 mM (twice the concentration of Ca present in Krebs solution) was examined: the response to eserine in 10^{-6} began to appear about 15 to 30 sec earlier than that with the control Krebs solution containing the same concentration of eserine, and always the size of sweat spots was larger than that produced by the control solution.

From these results it seems very likely to conclude that the concentration of Ca ions (2.5 mM) in Krebs solution is enough to contribute to the release of acetylcholine, and further that the increase in Ca ions facilitates the process of this release.

4) *Antagonism between Ca and Mg ions in the sudorific effect of eserine*

A total of 35 experiments were made for studying the effect of Mg ions on the sweat response to eserine. The eserine-containing test solutions were made in Krebs or in 0.9% NaCl solution to which CaCl_2 was added so as to contain Ca in 2.7 mM (almost equivalent to that in Krebs solution); MgCl_2 was added so as to contain Mg at concentrations of 1.2 to 53.8 mM. Control solutions were also used.

At concentrations of 1.2 mM (equivalent to the concentration of Mg ions in Krebs solution) and 3.5 mM, Mg ions showed no effect on the sweat response to eserine in 10^{-6} in all 13 tests except 2 tests in which a slight but definite inhibition of the response was observed. However, Mg in 4.6 mM (approximately four times the concentration of Mg in Krebs solution) showed always a definite inhibition. A more marked inhibitory effect of Mg ions was obtained at 23, 25, 52 and 53.8 mM (Fig. 4). The delay of the appearance of sweat spots on the injection wheals ranged from about 2 min and 30 sec to 16 min and 30 sec. Sweat spots were always small in number, as compared with those on the control wheals. For example, in one experiment about 120 sweat spots were counted on and around the injection wheal with a control solution containing eserine in 5×10^{-6} and extra NaCl osmotically equivalent to 25 mM Mg at 27 min after injection, while about 30 sweat spots were recognized on the wheal with the NaCl solution containing the same concentration of eserine and 25 mM Mg; and the number of spots increased to 180 on the former and 47 on the latter at 33 min after injection. Such inhibitory action of Mg ions was obtained in a total of 20 experiments testing the effects of Mg in 4.6 to 53.8 mM on the sweat response to eserine in 10^{-6} to 10^{-5} .

These results suggest that a concentration of Mg ions which is identical to that present in Krebs solution has no influence upon the release of acetylcholine from the sweat nerve endings even when it is increased still in subthreshold

Fig. 4. Inhibitory effect of Mg on the sweat response to eserine. Three wheals were produced, from left to right, by intradermal injections of 0.2 ml of 1) Krebs solution; 2) Krebs solution containing eserine in 10^{-6} ; and 3) Krebs solution containing eserine in 10^{-6} plus Mg in 53.8 mM. The injections were made at intervals of 37 and 35 sec respectively between the two successive injections, at 13°C dry bulb and 10°C wet bulb. At 4 min and 30 sec after the start of injection of the first solution, the subject entered a hot room at 29.5°C dry bulb and 21°C wet bulb, and took a cup of hot water.

Only on the wheal with the eserine-Krebs solution, sweating began to appear at 5 min and 25 sec after the start of its injection. Photographed at 25 min after the start of injection of the first solution. Note: no sweat responses are seen on the wheals with Krebs solution and with the Mg containing eserine-Krebs solution. $\times 1.5$.

amount, and that excessive Mg ions antagonize the action of Ca ions in the process of transmitter release.

5) *Effect of K ions on the sudorific effect of eserine*

A total of 16 experiments were performed to determine whether K ions have any influence upon the sweat response elicited by eserine in 10^{-7} and 10^{-6} . There was no significant difference in the responses to these concentrations of eserine no matter whether it was dissolved in KCl-free 0.9% NaCl or 4×10^{-4} KCl (5.4 mM K) containing 0.9% NaCl solution. This was confirmed with eserine containing test solutions to which CaCl_2 was added at 1.5×10^{-3} (13.5 mM Ca). These results show that K ions in a concentration as low as that present in Krebs solution (5.9 mM) might have no effect on the acetylcholine release induced under the present experimental conditions.

Further, when KCl was added at 10^{-3} (13.4 mM K), 4×10^{-3} (53.6 mM K) or 10^{-2} (133.8 mM K) to the eserine-containing or eserine-free NaCl solution, a more or less definite sweating developed on the injection wheal even at low environmental

temperatures. This response was more evident when KCl was added to Krebs solution containing no eserine, suggesting that KCl has a sudorific action on the sweat glands themselves in agreement with the description of Wada *et al.*⁵ An impression was obtained that the sudorific effect of K ions might be antagonized by Na ions. This may be probable, since one of us (K) found that when injected into the human skin the sudorific potency of KCl in 0.9% NaCl solution was less than that of KCl in an osmotically equivalent glucose solution.

6) *Effect of Mg ions on spontaneous thermoregulatory sweating*

In 32 experiments the effect of MgCl_2 injected intradermally was studied on spontaneous thermoregulatory sweating which was induced in a hot environment. Mg ions in Krebs solution were increased to 6.1, 16.0, 25.8, 50.4 and 99.6 mM by adding MgCl_2 in 28 experiments. In the other four experiments, MgCl_2 was added to 0.9% NaCl solution at 49.2 mM and 98.4 mM. In 9 of the 28 experiments testing the effect of MgCl_2 added Krebs solutions, control injections were made with Krebs solution and in the remaining 19 experiments they were made with Krebs solution to which glucose in amounts osmotically equivalent to those of MgCl_2 in test solutions was added. Likewise, in the 4 experiments testing the effect of MgCl_2 in 0.9% NaCl, control solutions were made by adding glucose, in place of MgCl_2 , to 0.9% NaCl.

About 1 to 4 min after completion of the injections, the test subjects were led to enter a hot environment at 38 to 43°C. A few minutes afterwards, thermoregulatory sweating commenced over the whole test areas. The effect of MgCl_2 was evaluated by comparing the time of the appearance of sweat spots and their density on the injection wheals produced by test solutions with those on the control wheals. Even when the concentration of Mg ions in Krebs solution was increased to 6.1 mM, the magnitude of thermogenic sweating did not show any notable difference from that on the control wheals in all 8 tests. But with Mg ions in 16.0 mM, a trace of inhibition of sweating was found in all 8 tests: the appearance of sweat spots was delayed for about 30 sec to 2 min, but the density of the spots became almost the same as that on the control wheal within about 10 min. A more evident inhibitory effect of Mg ions was seen with Krebs solution containing Mg at 25.8, 50.4 and 99.6 mM. At 50.4 mM, Mg delayed the appearance of response for 2 to 6 min, as compared with that on the control wheal, and about 10 to 20 min were required until the density of the spots became almost the same as that on the control wheal as well as in the areas surrounding each injection wheal. Further, the inhibition by Mg in 99.6 mM was more pronounced: sweat spots began to appear about 7 to 10 min later than those on the control wheal and about 20 to 30 min were required until the density of the spots on the wheal became equal to that on the control. Similar results were obtained with 0.9% NaCl solution containing Mg ions in 49.2 and 98.4 mM. With Mg ions in 49.2 mM tested in one experiment, the appearance of sweat spots was delayed for 2 min, as compared with that on a control wheal produced with glucose containing NaCl

solution and its inhibitory effect persisted for about 15 min. With Mg ions in 98.4 mM, the delay was for 2 to 6 min and their inhibitory effect subsided about 25 to 30 min after injection.

7) *Antagonism between the effects of Mg and Ca ions on spontaneous thermoregulatory sweating*

In 12 experiments the effect of Ca ions on the inhibitory effect of Mg ions in 26.2 mM was studied. Three injection wheals were made 1) with Krebs solution containing Mg in 26.2 mM and Ca in 5 or 10 mM; 2) with Krebs solution containing Mg in 26.2 mM and glucose at a concentration osmotically equivalent to Ca in 5 or 10 mM; and 3) with Krebs solution containing glucose in a concentration osmotically equivalent to Mg in 26.2 mM plus Ca in 5 or 10 mM. These injections were made soon after or at 2 to 5 min before the subject entered a hot room.

In these 12 experiments, Krebs solution containing Mg in 26.2 mM delayed the commencement of spontaneous thermoregulatory sweating on the injection wheal for 1 min and 30 sec to 4 min and 30 sec, as compared with that produced by the glucose added control Krebs solution; the central area of the wheals with Mg ion 26.2 mM was found to be devoid of sweat spots or their density was very low at the end of about 10 to 20 min after injection.

In 6 of 12 experiments with Krebs solution containing Ca in 5 mM and Mg in 26.2 mM, the latent period before the onset of sweating on the injection wheal was shortened for 2 to 9 min, as compared with that on the wheal with Krebs solution containing Mg in 26.2 mM plus glucose; the inhibitory effect almost subsided within 10 min after injection in a half of 6 tests, but it continued in another half still for 14 min or more after injection. The effect of Krebs solution containing Mg in 26.2 mM plus Ca in 5 mM was found to be intermediate between the effect of Krebs solution containing Mg in 26.2 mM plus glucose and the effect of Krebs solution to which glucose only was added (Fig. 5).

In contrast, on the wheal with Krebs solution containing Mg in 26.2 mM plus Ca in 10 mM in another 6 experiments, sweat spots became visible at almost the same time in 4 to 6 tests as on the wheal with the glucose added Krebs solution, and furthermore the wheal became full with sweat spots at almost the same time as on the control wheal with glucose added Krebs solution. In the remaining 2 tests, the appearance of sweating was delayed for 45 sec and 1 min and 20 sec respectively, the magnitude of sweating showed no significant difference from that on the control wheal within a few minutes. Thus, the results obtained indicate that the inhibitory effect of Mg in 26.2 mM on spontaneous thermoregulatory sweating was antagonized almost completely or very strongly by Ca in 10 mM.

These results indicate that the antagonizing effect of Ca towards the inhibitory effect of Mg on spontaneous thermoregulatory sweating was almost complete in a ratio of 1:2.6 in molality, but it was incomplete in a ratio of 1:5.2. In the above-described experiments with eserine, the action of Ca ions in 2.7 mM on the acetylcholine release from the sweat nerve endings was definitely inhibited by Mg

Fig. 5. Effects of Mg and Ca on spontaneous thermoregulatory sweating. Three wheals were produced, from left to right, by intradermal injections of 0.2 ml of 1) Krebs solution containing Mg in 26.2 mM and Ca in 5 mM; 2) Krebs solution containing Mg in 26.2 mM; and 3) Krebs solution only. The second and third solutions were made osmotically equal to the first solution by adding glucose. The injections were made at intervals of 26 and 25 sec respectively between the two successive injections, at 36.5°C dry bulb and 30.5°C wet bulb.

Spontaneous thermoregulatory sweating began to appear over the test area outside the three wheals at 4 min and 15 sec after the start of injection of the first solution. On the wheal with the glucose added Krebs solution, sweat spots began to appear at 4 min and 40 sec after the start of its injection; and on the wheal with Krebs solution containing excessive Mg and Ca, they became visible at 6 min and 45 sec after the start of its injection.

Photographed at 12 min and 50 sec after the start of injection of the first solution. Note: the wheal with Krebs solution containing excessive Mg is devoid of sweat spots. $\times 1.2$.

in 4.6 mM; that is, in a ratio of 1:1.7 in molality. From these results, the facilitating effect of Ca on the acetylcholine release seems to be antagonized by Mg in a ratio of approximately 1:2 in molality.

8) *Effect of Mg ions on the responsiveness of the sweat glands to acetylcholine and mecholyl*

In 6 experiments three injection wheals were made 1) with 0.9% NaCl solution containing acetylcholine in 10^{-10} plus Mg ions in 49.2 mM; 2) with 0.9% NaCl solution containing acetylcholine in 10^{-10} only; 3) with 0.9% NaCl solution containing acetylcholine in 10^{-10} and glucose in a concentration osmotically equivalent to Mg in 49.2 mM. The NaCl solution containing acetylcholine plus Mg, when injected intradermally, showed a definite sweat response which began to appear 40 to 70 sec, 53 sec on an average, comparable to 40 to 60 sec, 51 sec on an

average obtained with the NaCl solution containing acetylcholine only. In the tests with the saline solution containing acetylcholine plus glucose, the onset of the sweat response was somewhat delayed. In general, the response to acetylcholine in 10^{-10} ceased within about 10 to 15 min after injection.

Similarly, in additional 4 experiments there was no evidence to indicate any inhibitory effect of Mg in 24.6 mM on the local sweat response to acetylcholine in 10^{-8} and in 10^{-11} .

In 8 experiments, the effect of Mg on local sweating produced by intradermal injection of mecholyl in 10^{-8} was examined in the same way as described above. The NaCl solution containing mecholyl in 10^{-8} and Mg in 49.2 mM caused a local sweating within 1.5 to 2 min after injection. This latent period was comparable to 1 min and 10 sec to 3 min, 1 min and 49 sec on an average, obtained with the NaCl solution containing mecholyl in 10^{-8} alone. No inhibitory effect was found with Mg in 49.2 mM, when the NaCl solution containing Mg plus glucose was used as control. In further 6 experiments, Krebs solution containing Mg in 50.4 mM plus mecholyl in 10^{-8} showed no significant difference in the latent period before the appearance of response from Krebs solution containing mecholyl in 10^{-8} plus glucose in a concentration osmotically equivalent to Mg in 50.4 mM. It may be noted that the sweat response to mecholyl in 10^{-8} subsided within about 15 to 25 min after injection.

These results demonstrated that Mg ions, even when increased up to 50.4 mM, did not reduce the excitability of the sweat glands. This shows that at such a high concentration, Mg does not reduce the responsiveness of the sweat glands to acetylcholine released from the sweat nerve endings. Therefore, it may be reasonable to conclude that the above-described inhibitory action of Mg ions is localized at the nerve endings from which acetylcholine is liberated.

DISCUSSION

Janowitz and Grossman⁶ found that eserine in 10^{-3} elicited a local sweating when injected intradermally to the human forearm, and assumed that acetylcholine is constantly released from the sweat nerve endings in subthreshold amounts and is built up above threshold levels by the anti-cholinesterase activity of this concentration of eserine. Randall and Kimura⁷ described shortly the experience of one of them that eserine which had been ineffective in eliciting sweat response at room temperature of 20°C for a prolonged period became effective when entering a warm room at 33°C.

In the present experiments, it was demonstrated that eserine in a concentration as low as 10^{-8} or 10^{-7} became effective in provoking a local sweating when the subject entered a warm environment. When its concentration was raised up to 10^{-6} or 10^{-5} , eserine was found frequently to be effective even in a cool environment, but with a longer latent period after injection. However, the response to such higher concentrations of eserine was intensified in a warm or hot environment. These results provide indirect but good evidence indicating that the quantal

release of acetylcholine from the sweat nerve endings is increased on exposure to heat, even though it is still below the threshold for inducing the sweat response. However, it is not clear whether the human sweat glands receive a tonic innervation, and then whether acetylcholine is being liberated always constantly even when sweating is absent. At any rate, the sudorific effect of eserine in low concentrations such as 10^{-8} and 10^{-7} in a warm environment seemed to depend entirely upon the amount of acetylcholine released from the sweat nerve endings. The same may be true of the sudorific effect of eserine in higher concentrations. But, the possibility that eserine acts in part directly on the sweat glands themselves at higher concentrations cannot be taken out of consideration, as pointed out by Langley and Kato.⁸

As described above, a low concentration of procaine such as 10^{-4} inhibited definitely the sudorific effect of eserine in 10^{-7} , suggesting that this concentration of procaine is sufficient to interfere with the release of a subthreshold amount of acetylcholine in a warm environment. In order to suppress spontaneous thermoregulatory sweating, the concentration of procaine must be increased up to 10^{-3} . From this, it is evident that the release of acetylcholine in subthreshold amounts is easily affected with a lower concentration of procaine, as compared with that for the release of acetylcholine in threshold or superthreshold amounts.

The present results show that Ca ions in a concentration comparable to that contained in Krebs solution (2.5 mM) was found to be essential to the process of release of acetylcholine, especially when it was released in subthreshold amounts. This may be true of Ca ions in the extracellular fluid. However, the presense of Ca ions in 5 to 45 mM seemed to facilitate the acetylcholine release. That the action of Ca is concerned directly with the acetylcholine release was established recently by Katz and Miledi⁹ who obtained increased release of acetylcholine by electrophoretic application of Ca to the frog's motor nerve terminals.

In contrast to Ca ions, the concentration of Mg and of K ions present in Krebs solution (1.2 and 5.9 mM, respectively) was found to have no influence upon the release of subthreshold amounts of acetylcholine. At a concentration of 4.6 mM (about four times the concentration of Mg in Krebs solution) or more, Mg ions inhibited the release of acetylcholine very probably by antagonizing the effect of Ca ions. On the other hand, when the concentration of K ions was raised up to 13.4 mM or more, they were found to stimulate directly the sweat glands themselves.

For locally inhibiting spontaneous thermoregulatory sweating in a hot environment, the concentration of Mg ions must be increased to 25.8 mM or more. This effect of Mg ions might be largely due to their inhibitory action on the acetylcholine release, since the responsiveness of the sweat glands to exogenous acetylcholine was not affected by Mg ions in a concentration as high as 49.2 mM.

The inhibitory effect of Mg ions in 26.2 mM on spontaneous thermoregulatory sweating was antagonized almost completely by Ca in 10 mM. From the present results, the antagonism between Ca and Mg ions may be complete in a ratio of approximately 1:2 in molality. A similar findings was obtained by Hutter and

Kostial:^{10,11} Mg in 15–25 mM reduced the acetylcholine release from the preganglionic sympathetic nerve endings in the perfused superior cervical ganglion of the cat, and Ca restored this release at 4–10 mM. Furthermore, the present results are in line with the finding of Katz and Miledi¹² that in an equimolar concentration such as 1 M, Mg is less potent than its antagonist Ca in releasing acetylcholine from the motor nerve endings in the frog.

Finally, it may be pointed out that the responsiveness to nicotine and NaCl of the receptors for the sweating axon reflex, which have been presumed to be specialized portions of the cholinergic sweat nerve endings, is reduced or abolished in the presence of excessive Ca,^{5,13} in contrast to its facilitating effect on the acetylcholine release from the sweat nerve endings.

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