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Influence of Calcium-Deficit on Morphine Inhibition of QO_2 of Rat Cerebral Cortex Slices.* (28569)

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There is general agreement that morphine has little effect on the metabolism of brain tissue respiring in the usual Ringer-type solutions. Concentrations as high as 0.01 M had no effect on the QO_2 of rat brain cortex slices respiring in glucose-Ringer's(1), and 0.0056 M was found not to affect glycolysis by rat brain homogenates(2). However, with special media and with certain substrates, inhibition of oxygen uptake by brain tissue preparations has been reported. Quastel and Wheatley(3) worked with chopped whole guinea pig brain suspended in phosphate buffer pH 7.4 containing 0.6% saline. They found that 0.12% (0.0016 M) morphine inhibited the increment in oxygen uptake caused by the addition after 3 hours of glucose, lactate, pyruvate and glutamate, but not succinate or p-phenylenediamine. Seevers and Shideman(4) reported that 0.12% morphine inhibited by 10-30% the QO_2 of rat brain slices in Ringer M/60 phosphate buffer pH 7.35 when the substrate was lactate, pyruvate, or α-ketoglutarate, but not when the substrate was glucose, succinate, fumarate, or malate.

Recently Bell(5) found that the stimulative response of rat cerebral cortex in Krebs-Ringer phosphate medium to applied electrical pulses was partially inhibited by 0.0001 M morphine and practically abolished by 0.001 M morphine. Takemori(6), using a Ringer's solution low in calcium (0.0005 M) reported that 0.001 M morphine inhibited the oxygen uptake of potassium stimulated cerebral cortex slices from control rats, but not from rats tolerant to daily doses of 90/mg/kg of morphine. He later reported(7) the same results with a medium containing the usual amount of calcium (0.0013 M). He also stated that nalorphine reversed the apparent adaptation of brain slices to morphine and claimed(8) complete cross-cellular adaptation with methadone in cortical slices adapted to the depressive effect of morphine.

During a study of the effects of cold exposure on some actions of morphine we attempted to duplicate Takemori's basic observation that morphine could inhibit potassium stimulated oxygen uptake of rat cerebral cortex slices. We were unable to show any inhibitory effect of 0.001 M or 0.002 M morphine on brain slices from control rats and from rats exposed for one month to an ambient temperature of 4°C. In an effort to account for this discrepancy, we noted that Takemori(6) had originally used a low-calcium Ringer's solution and therefore repeated the experiments using calcium-free

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Ringer's solution. With this medium we were able to demonstrate inhibition of potassium-stimulated oxygen uptake by brain slices from control rats, but not from rats tolerant to 100 mg/kg/day of morphine. Our reasons for believing that the effects of morphine on potassium-stimulated brain slice respiration occur in calcium-free Ringer's but not in conventional Ringer's are detailed below.

Methods. The animals used in these studies were young adult male Long-Evans rats, or, in one experiment, Holtzman rats. Controls were taken directly from the animal room. Rats were made tolerant to morphine by daily administration of morphine sulfate intraperitoneally in increasing doses over approximately one month until they were tolerant to 100/mg/kg/day. When used, their general condition was good, as evidenced by continuing weight gain. Cold exposed rats lived 2 in a cage for one month at an ambient temperature of approximately 4°C.

Brains were obtained by decapitation and rapid dissection. They were transferred immediately to a moist cold box where cortical slices approximately 0.5 mm thick were prepared with razor blade and template. Usually as much gray matter as possible was sectioned, but in some cases surface slices were separated from the remainder of the tissue. Approximately 40 mg samples were weighed on a torsion balance and transferred to 0.9 ml of chilled suspending media in previously prepared Warburg flasks. Oxygen consumption was measured by the direct method of Warburg at 37°C. The gas phase was oxygen. Carbon dioxide was absorbed by 0.2 ml of 10% KOH in the center well. The initial manometer readings were made less than 30 minutes after decapitation. Control readings were then made every 10 min for 50 minutes, after which KCl, morphine, or both were added from the sidearm, and readings continued for 60 minutes. The usual medium contained NaCl 0.145 M, KCl 0.004 M, $CaCl_2$ 0.0016 M, $MgSO_4$ 0.0011 M, Na_2HPO_4 phosphate buffer 0.01 M pH 7.35 and glucose 0.012 M. In one experiment the Ringer's solution described by Takemori(7) was used, made up from salts obtained from his supplier. When potassium-stimulation was desired, 1.0 M solution was added from the sidearm to give a final concentration of 0.1 M. Morphine SO_4 was added in the same manner to give a final concentration of 0.002 M. Rate plots were made of QO_2 values calculated on a wet weight basis, and possible significance among experimental results was determined by analysis of variance according to the method of Scheffé(9) using an IBM 1620 computer. With the statistical methods used, a 10% level of significance is approximately equal to a 5% level obtained by conventional t tests between any pair of values.

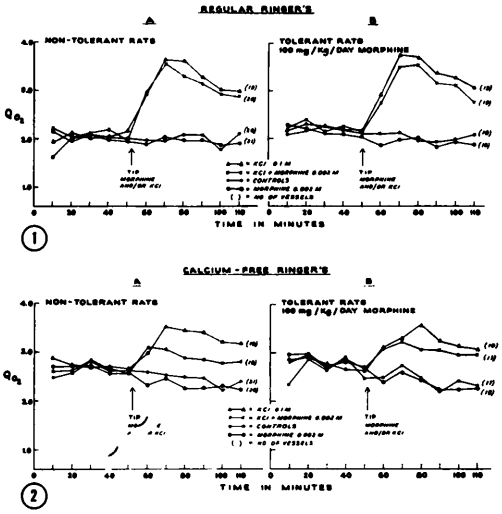

FIG. 1. Lack of effect of 0.002 M morphine on oxygen uptake of rat brain slices in regular Ringer's with and without potassium stimulation. A. Control rats. B. Rats tolerant to morphine 100 mg/kg/day. In both cases there is no significant effect on potassium stimulated respiration at 70, 80, 90, 100, and 110 min.

FIG. 2. A. Effect of 0.002 M morphine on oxygen uptake of rat brain slices in calcium-free Ringer's with and without potassium stimulation. Inhibition of potassium-stimulated respiration by morphine was significant at the following levels between 70 and 110 min. 70 min = 5%, 80 min = 1%, 90 min = 10%, 100 min = 10%, 110 min = 5%. B. As for A using brain slices from rats tolerant to morphine 100 mg/kg/day. There was no significant inhibition of potassium stimulated respiration by morphine except at 80 min (10% level).

Results. Our findings using conventional Ringer's solution are illustrated in Fig. 1A and B. A QO_2 of approximately 2.0 was maintained for 110 minutes by cerebral cortex slices from controls and from rats tolerant to daily doses of 100 mg/kg of morphine. In

neither case did the addition of morphine in final concentration of 0.002 M significantly affect the values. Addition of KCl in final concentration of 0.1 M after the 50 minute reading increased the QO_2 to about 3.6 within 20 minutes. The rate then decreased slightly over the next 40 minutes. When morphine was added with the KCl the rates were not significantly decreased for slices obtained from control animals. Slices from morphine-tolerant rats were likewise unaffected. When these experiments were repeated with a group of 10 cold exposed rats precisely the same results were obtained.

Since our findings were at variance with those of Takemori(7,8), we obtained Holtzman rats such as he used. In experiments utilizing 20 tissue samples from 4 rats, addition of either KCl or KCl and morphine produced the same effect as illustrated in Fig. 1A. Also, morphine did not inhibit the potassium-stimulated oxygen uptake of brain slices from Holtzman rats when our Ringer's solution was used. Furthermore, the oxygen uptake of surface slices such as those used by Takemori and the effects of KCl and morphine thereon did not differ from results obtained with tissue samples made up from all layers of the cerebral cortex. Thus even though the conditions of Takemori's experiments were duplicated as closely as possible in different laboratories, we were unable to reproduce his results.

However, when calcium-free Ringer's was substituted for the usual suspending medium, entirely different findings emerged (Fig. 2A and B). As expected, QO_2 values were higher and 0.002 M morphine had no effect on oxygen uptake of brain slices from either control or morphine-tolerant rats. When added at 50 minutes 0.1 M KCl elevated the QO_2 of slices from control rats to the same value obtained in regular Ringer's. When KCl and morphine were added simultaneously the oxygen uptake of slices from control rats was significantly inhibited (Fig. 2A). These results were verified with brain slices from cold exposed rats. In this series, not illustrated, the inhibition was significant at the 5% level during the 70-110 minute interval. Morphine (Fig. 2B), failed to inhibit significantly the oxygen uptake of brain slices from rats tolerant to daily doses of morphine 100/mg/kg. While the reversal of inhibition is not complete, these results are in line with those of Takemori.

Discussion. The absence of an effect of morphine on oxygen uptake of rat cerebral cortex slices respiring in conventional Ringer's solutions was not an unexpected finding in view of past experience. However, inhibition of the potassium-stimulated QO_2 in calcium-free Ringer's solutions is of special interest because it implicates ions of known neurophysiological and neurochemical importance. Furthermore, even though the concentration of morphine required to produce inhibition is relatively high, the inhibition is partially reversed by the development of tolerance to morphine. As pointed out by Takemori(6), this may exemplify an adaptation to morphine at the cellular level representative of adaptation of the whole rat to morphine. We could not duplicate Takemori's results(7,8) with carefully controlled experiments although the morphine effect demonstrated in calcium-free Ringer's was readily repeatable with tissue from a group of cold exposed rats. Our findings have been confirmed by D. Rosenberg (personal communication) and by D. Clouet (personal communication). Neither of these investigators, working in different laboratories, was able to show any effect of morphine on the potassium stimulated QO_2 of rat brain slices in conventional Ringer's.

Adequate explanation for our own findings may well await elucidation of the still obscure reasons for the stimulation of oxygen uptake of brain slices in calcium-free Ringer's. This has been assumed(10,11) to be related to alteration of the K^+/Ca^{++} balance, but we are unaware of any direct evidence to indicate that the stimulation seen with calcium-free Ringer's is merely a lesser degree of potassium stimulation. If this were so, morphine might be expected to inhibit in calcium-free Ringer's *per se*, whereas actually it has no appreciable effect except in the presence of a high concentration of potassium.

Since calcium may affect the electrical ac-

tivity of nerve tissue by virtue of its action at the cell membrane or it may affect neuronal metabolism by an action on one or more steps of the phosphate transfer system (12), both surface and metabolic effects must be considered in attempting an explanation of the actions of morphine in calcium-free Ringer's. Current experiments in our laboratory are concerned with possible changes in cell permeability due to calcium deficit as well as with the effects of morphine on the metabolism of various rat tissues and in the presence of substrates other than glucose.

Summary. Morphine (0.002 M) has been found to inhibit significantly the potassium-stimulated oxygen uptake of rat cerebral cortex slices respiring in calcium-free glucose-Ringer's solutions. This effect is virtually absent with brain slices made from rats tolerant to 100 mg/kg/day of morphine. Inhibition by morphine does not occur in conventional Ringer's solution, indicating that the effect is linked to the ionic constitution of the suspending medium. Exposure of rats to an environmental temperature of 4°C for one month does not alter the effect of morphine.

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