Pharmacology Biochemistry & Behavior, Vol. 3, pp. 915–918.

BRIEF COMMUNICATION

Feeding Following Intraventricular Injection of CA^{++},MG^{++} or Pentobarbital in Pigs

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(Received 26 December 1974)

BALDWIN, B. A., W. L. GROVUM, C. A. BAILE AND J. R. BROBECK. *Feeding following intraventricular injection of Ca^{++}, Mg^{++} or pentobarbital in pigs.* PHARMAC. BIOCHEM. BEHAV. 3(5) 915–918, 1975. – The effects on food intake, of injections into the lateral ventricle of Ca^{++}, Mg^{++} or equimolar mixtures of the 2 ions have been studied in pigs. The chloride salts were made up in normal saline which together with 1.35 percent NaCl was used as a control injection. Dosages of 12.5 μmoles, 25 μmoles, or 50 μmoles either Ca^{++} or Mg^{++} elicited increased food intake in the 30 min following the injection and there was a predominantly linear feeding response to increasing concentration of the two ions. Ca^{++} was more effective in eliciting increased food intake than Mg^{++}. Equimolar mixtures of Ca^{++} and Mg^{++} of 6.5 + 6.5 μmoles, 12.5 + 12.5 μmoles or 25 + 25 μmoles also increased food intake linearly and the mixture of ions was more effective than equivalent concentrations of Ca^{++} or Mg^{++} alone. No antagonism between Ca^{++} and Mg^{++} was seen with respect to food intake. Intraventricular injections of 16 μmoles of sodium pentobarbital also elicited increased food intake in the 30 min following injection. It is suggested that the ions and the barbiturate probably act by depression of periventricular neurons, particularly in the medial hypothalamus which is adjacent to the third ventricle.

Intraventricular injection Feeding Pentobarbital Ca^{++} Mg^{++}

THE application of chemical substances via the intraventricular route, now a widely used method for the study of many aspects of neurophysiology and behavior [3,11], avoids the blood brain barrier [21] and permits the use of low concentrations of the injected substance. Recently, the effect on feeding behavior of intraventricular injections of calcium, magnesium, sodium or potassium ions has been studied. In rats, elevation of the concentration of Ca^{++} in the cerebrospinal fluid (CSF) by injection into the lateral ventricle resulted in voracious feeding [12,14]. Raising the concentration of Ca^{++} or Mg^{++} in the CSF of sheep, by injection into the third ventricle, resulted in increased food intake which was related to the dose administered [18].

In the present experiments we have examined the effects on food intake in the pig of injection of solutions containing calcium or magnesium chloride into the lateral ventricle. We have also ascertained the effect on food intake of intraventricular injection of mixtures of Ca^{++} and Mg^{++} in order to establish whether, with respect to feeding behavior, they act in an antagonistic manner, as it is well known that the depressant effects of magnesium salts injected intravenously can be counteracted by injection of calcium salts [22].

In addition to experiments in which calcium or magnesium ions were injected, we have also examined the effects on food intake of intraventricular injection of pentobarbital, which has previously been shown to cause feeding when injected intraventricularly in sheep [15], goats [1], cattle [15] and rats [2,10].

METHOD

Animals

Experiments were carried out on 6 pigs of the Yorkshire breed aged about 10–12 weeks at the start of the experiments. The pigs were sexually immature females. They were

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housed individually in metal cages in which food and water were continuously available. The food consisted of a pelleted ration which contained 17 percent protein. The temperature of the animal room was maintained in the range 20–25°C and the room was illuminated from 7 a.m. to 5 p.m.

Procedure

All surgery was performed using general anesthesia which was induced with Halothane using a face mask, and following induction the pigs were intubated with a cuffed endotrachael tube and maintained on Halothane using a closed circuit apparatus. Antibiotics were routinely administered after the operations. At least 3 weeks elapsed between the surgery and the first experiments.

The pigs were provided with bilateral cannulae guides directed towards the lateral ventricles. The guides were made from 18 ga stainless steel needles which were cut so that they were 14 mm long from the tip of the needle to the base of the hub. A midline incision was made and the surface of the skull was exposed about 3 cm each side of the bregma. Holes about 1.5 mm in diameter were drilled through the skull at points 1 cm lateral to the bregma along the line of the fronto-parietal suture. The holes were drilled at right angles to the surface of the skull and the dura pierced using a sharp needle. The guides were inserted into the skull one on each side of bregma and fixed in position using dental cement. Four self-tapping stainless steel screws (No. 4 X 7 mm) were inserted into the skull to secure the cement to the skull and the guides were protected by means of 2.5 cm of a stainless steel cylinder (3.7 cm dia.) in a manner similar to the plastic caps previously described for use in sheep [17]. The guides were closed by means of a stainless steel stylet. The insert for ventricular injections was a 22 ga needle passed through a hole drilled lengthwise in a male Luer plug and held in place by a side-screw so that the length of insert in the brain could be adjusted. The correct depth for the insert to enter the lateral ventricle in each pig was determined by adjusting the depth until (a) saline in tubing connected to the insert would flow freely in when the tubing was held above the pig's head and (b) CSF would drip out when the end of the tubing was held below the level of the pig's head.

During the experiments all intraventricular injections were 1 ml in volume and all solutions used were passed through a 0.22 μ Millipore filter. At least 48 hr elapsed between injections. The patency of the cannula inserted into the lateral ventricle was checked before each injection by noting whether the solution to be injected would run into the ventricle with 15 to 30 cm of water pressure. All solutions of magnesium chloride or calcium chloride were made up in normal saline and the 1 ml was injected in about 30 sec. All 6 pigs received the same injection on any given occasion but the various concentrations and substances were given in a random order. An attempt was made to vary the injections between the two ventricles in individual animals.

The experiments were carried out as follows. At about 9 a.m. fresh food was added to each pig's food container; 90 min later the injections were given and the amount of food eaten in the 30 min following the injection was recorded. Usually the pigs were lying and resting in their cages before the injections were given.

RESULTS

Following injection of 1 ml of solutions of magnesium chloride, calcium chloride or equimolar mixtures of the two salts the pigs usually started to eat within 1 or 2 min of receiving the injection. They terminated their feeding within 30 min of the injection and lay down and apparently slept but were easily aroused if disturbed. The injections did not cause ataxia or any obvious signs of sedation, excitement or discomfort and the feeding resembled that seen in a normally hungry pig. As shown in Fig. 1 there was very little food intake in the 30 min following the injection of 1.35 percent NaCl or 0.9 percent NaCl used as control injections. The 1.35 percent NaCl was approximately isosmotic with the most concentrated solutions of calcium or magnesium.

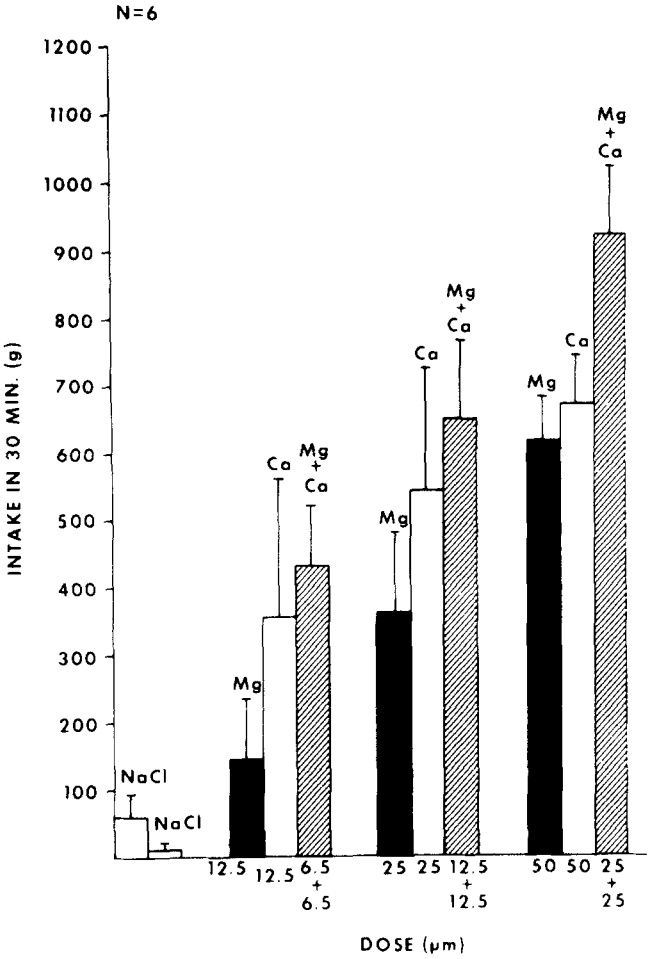

FIG. 1. The relation between the dose of calcium, magnesium or calcium plus magnesium and the amount of food eaten in the 30 min following the intraventricular injection. Bars indicate SEM.

As displayed in Fig. 1, increasing the dose of Mg^{++} increased the amount of food eaten and the same was true for Ca^{++} or equimolar mixtures of the two ions. Statistical analysis of the data revealed that, within the ranges of concentration used, there was a predominantly linear response to increased concentration of calcium, magnesium or calcium plus magnesium ions ($p<0.01$). The analysis showed that

the Ca^{++} plus Mg^{++} combination was more effective than Mg^{++} alone ($p<0.05$); but in comparison of the effect of Mg^{++} plus Ca^{++} versus Ca^{++} the difference fell short of the 5 percent level of significance. Ca^{++} alone, however, was significantly more effective than Mg^{++} alone ($p<0.05$).

In addition to the injections of Ca^{++} and Mg^{++}, 12 observations were made on the effects of injecting 16 μmoles of sodium pentobarbital in 1 ml of normal saline. The procedure did not cause obvious ataxia and the pigs consumed an average of 433 ± 33 g in the 30 min following the injection. This intake compares with a maximum average intake of 61 ± 34 g when the saline controls were injected. Following injections of pentobarbital the pigs were usually feeding within 1 or 2 min of the injection.

A limited number of observations was made on the intake of food during 24 hr periods. It was not possible, with the facilities available, to do this routinely as the pigs frequently contaminated with feces or spilled their food. Nevertheless, in two observations made of the food intake of each pig in a 24 hr period the mean intake was 1901 ± 127 g.

DISCUSSION

Intraventricular injection of either Ca^{++} or Mg^{++} or both elicited feeding in pigs which had had continuous access to food in the period preceding the injection; however, Ca^{++} caused slightly more food to be eaten than did Mg^{++} at equal doses. These results confirm and extend previous findings in sheep in which injections of these cations into the third ventricle elicited feeding [18]. Feeding following Mg^{++} injections into pigs and sheep contrasts with the lack of response reported in the rat following Mg^{++} injections into the lateral ventricle, although Ca^{++} has elicited feeding in all three species [12,14].

In the present experiments, administration of Ca^{++} or Mg^{++} into the lateral ventricle does not permit firm conclusions to be drawn about the site of action of the cations although the work on sheep, using the third ventricle as the injection site, suggests that the cations were acting on the periventricular regions of the hypothalamus. Myers and Veale showed, using cats, that feeding can be induced by push-pull perfusion of localized regions of the hypothalamus with solutions containing excess calcium ions [13]. In their experiments, perfusion of a site in the ventromedial region produced a larger food intake than perfusion of sites in the dorsal or ventral regions of the lateral hypothalamus. In experiments with sheep, hypothalamic injections of Ca^{++} or Mg^{++} in anterior, ventromedial, dorsomedial, posterior or lateral hypothalamus elicited feeding [19].

One possible explanation of the present results is that both Ca^{++} and Mg^{++} are acting by depressing the function of medial regions of the hypothalamus and reducing their inhibitory actions on the lateral hypothalamus [4]. It is known that small amounts of Ca^{++} or Mg^{++}, applied by means of micro-iontophoresis, have a markedly depressant effect on neuronal firing in the cerebral cortex and other parts of the brain [6, 7, 16]. Phillis [16], in a recent review, considers that externally applied Ca^{++} may depress neurons by interfering with the increase in sodium permeability which generates the action potential. Krnjevic [8], reviewing the effects of iontophoretic application of substances upon cortical cells states that both Ca^{++} and Mg^{++} are relatively potent depressants, and it has been shown, with respect to cortical neurons, that Ca^{++} is a more potent depressant than Mg^{++} [7]. Inasmuch as we found (Fig. 1) that Ca^{++} induced more feeding than equimolar amounts of Mg^{++} this relationship tends to support the idea that the effect is produced by a mechanism dependent upon depression of neurones.

Apparent central depression and deficits in peripheral neuromuscular junctions caused by hypermagnesaemia can be completely reversed by injections of Ca^{++} although the mechanism of this antagonism has not been elucidated [22]. Intravenous injection of Mg^{++} does not result in general anesthesia because the excess Mg^{++} does not pass from the plasma to the brain; the immobilization is due entirely to peripheral paralysis at the neuromuscular junction [5,20]. In contrast to the antagonistic effects of Ca^{++} and Mg^{++} when administered intravenously, Leuson [9] has pointed out that, when injected directly into the CSF, the depressant effects of increased concentrations of both ions are cumulative. He also states that the effects of Ca^{++} and Mg^{++} are possibly due to action on the neuronal membrane rather than interference with synaptic transmission and that both ions have a stabilizing effect on the neuronal membrane. Kato and Somjen [6], on the basis of experiments using iontophoretic application of Ca^{++} and Mg^{++} to neurons, stated that the combined depressant effect of the two ions was synergistic and there was no evidence of competition between them.

In our experiments, judging by the feeding response, there was no indication of any antagonistic action between Ca^{++} and Mg^{++} when equimolar mixtures were injected. As may be seen from Fig. 1 the food intake induced by the mixture was greater than equivalent concentrations of either cation alone, which fits in well with the electrophysiological data.

With regard to the possible role of fluctuations in Ca^{++} and Mg^{++} concentrations in the CSF as factors influencing the pattern of food intake, it is known that the concentration of these ions shows a remarkable stability even when blood levels are acutely or permanently altered [9]. It therefore seems unlikely that alterations in the concentration of these ions in the CSF plays a part in the normal regulation of food intake. However, the reliable manner in which increases in the concentrations of these ions in the CSF induces dose-related feeding should prove a useful method for the study of the normal control of food intake.

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