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THE LOCATION OF THE CHEMORECEPTOR CONTROLLING GASTRIC ACID SECRETION DURING HYPOGLYCAEMIA

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SUMMARY

1. The injection of 2-deoxy-D-glucose directly into the lateral hypothalamic area of rats, but not elsewhere, caused a prompt and sustained secretion of acid by the stomach at a rate comparable to that due to insulin hypoglycaemia.

2. Acid secretion provoked by such injections, like that resulting from hypoglycaemia, could be stopped by raising the plasma glucose concentration by the intravenous infusion of glucose.

3. Unilateral intrahypothalamic injection of 2-deoxy-D-glucose activated both vagi for, although cutting one vagus reduced the secretion, division of both was necessary to abolish it.

4. Gastric acid secretion evoked by a systemic stimulus (insulin-induced hypoglycaemia or intravenous 3-*O*-methylglucose) could be prevented by inactivating the lateral hypothalamic area on each side with phenol or lignocaine.

5. It is concluded that there exists in the lateral hypothalamic area a chemoreceptor, responsive to a lack of metabolizable glucose, which can initiate and sustain the vagally mediated secretion of acid by the stomach.

INTRODUCTION

When the plasma glucose concentration falls below a critical level, as in insulin-induced hypoglycaemia, the stomach is stimulated to secrete acid. Gastric acid secretion may also be caused by the administration of two synthetic glucose analogues, 2-deoxy-D-glucose (Hirschowitz & Sachs, 1965) and 3-*O*-methylglucose (Colin-Jones & Himsworth, 1969). It is thought that in all these cases secretion is essentially due to a diminished availability of glucose for metabolism by a tissue which is particularly

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sensitive to such a lack. Secretion caused by each of the three stimuli may be ended by cutting both vagi or by the administration of glucose (Colin-Jones & Himsworth, 1969; Himsworth & Colin-Jones, 1970). It is believed, on the basis of these similarities, that the same mechanism is activated by each stimulus to bring about the increased gastric acid output and that the response is determined by specific chemoreceptors. The exact location of these hypothetical chemoreceptors is unknown although cross-circulation experiments by La Barre & de Cespedes (1931) showed that the gastric secretory response to hypoglycaemia arises from somewhere within the vascular territory supplied by the common carotid artery. As decerebration prevents the secretion of acid during hypoglycaemia whereas decortication does not (Jögi, Ström & Uvnäs, 1949) it is probable that the hypothalamus participates in the response and even that the impulse to secretion may originate from this part of the brain.

In the present state of knowledge there is little information about the existence and position of such chemoreceptors in the substance of the central nervous system. Such knowledge as we possess has been largely derived by making discrete lesions in or electrically stimulating the brain. In the former case the results are open to the criticism that the effects might be influenced by the relatively major trauma produced. In the latter case there is always the doubt as to the physiological significance of the effects of such stimulation. We therefore attempted to locate the position of the hypothetical chemoreceptors by measuring the rate of gastric acid secretion while producing a local inhibition of glucose metabolism within different parts of the brain by the injection of small amounts of 2-deoxy-D-glucose. In thus attempting to fix the position of a chemoreceptor in the central nervous system by direct and specific chemical provocation it appears necessary to satisfy the following five general postulates. 1. An agent which activates a chemoreceptor controlling a physiological function when administered systemically should produce the same effect when applied in a relatively smaller dose to the alleged site of the chemoreceptor in the brain. 2. Application of the agent to all other parts of the brain should not produce such an effect. 3. The response to local application within the brain or by systemic administration should be mediated by the same nervous pathways. 4. If the action of the agent producing a positive response can be reversed by other substances these should be effective whether the agent be locally applied to the chemoreceptor or systemically administered. 5. Inactivation of the area of the brain which is sensitive to the local application of a provocative agent should abolish the response to a systemically delivered stimulus. Unless all of these five postulates are satisfied the evidence for the existence and localization of a chemoreceptor, although it may be highly presumptive, cannot be conclusive.

METHODS

103 male Norwegian hooded rats weighing between 200 and 300 g were used in these experiments. Preparation, anaesthesia and operative techniques (insertion of cannulae and inactivation of the visceral sympathetic nervous system) were the same as those described in an earlier paper and the same method was used to record the gastric acid output (Colin-Jones & Himsworth, 1969). After being prepared the rat was mounted in a conventional stereotaxic apparatus (Baltimore Instrument Company) in such a position that it was possible to record continuously the secretion of acid by the stomach while operating upon the head. The skull position, system of stereotaxic co-ordinates and anatomical nomenclature described by Szentágothai, Flerkó, Mess & Halász (1968) were followed in all experiments. Intrahypothalamic injections were made with a microsyringe ('Agla', Wellcome) connected by fine polyethylene tubing to a blunt steel cannula (o.d. 0·5 mm) which was mounted on the micromanipulator of the stereotaxic apparatus. The cannula was introduced into the substance of the brain through holes trephined in the vault of the skull. To make an injection, the cannula was inserted to the required depth, it was then removed and any fragments of brain were cleared from its lumen and finally it was lowered exactly along the previous track to the same position. Neither this procedure nor the preceding craniotomy caused any change in the negligible basal rate of acid secretion. The cannula was generally left *in situ* for at least 10 min after an injection had been made. The volume of any injection never exceeded 5 μl. In the initial experiments 2-deoxy-D-glucose was given as an isotonic solution in water, in later experiments it was dissolved, to the same concentration, in a buffer (pH 7·4) whose ionic composition approximated to that of the plasma: the medium used did not appear to influence the results obtained. Lignocaine and phenol were given as 2 % and 5 % (w/v) solutions in water respectively. At the end of every experiment the whole of the top of the skull was removed and the head was fixed for 48 hr in Heidenhain's Susa. The brain was then trimmed and mounted in wax. Sections (20 μ) were cut in the coronal plane through the hypothalamus and serially mounted. The sections were stained with cresyl violet and differentiated with colophonium.

Blood was taken from the tip of the tail for glucose estimation as described in a previous paper (Colin-Jones & Himsworth, 1969). Plasma glucose was estimated either by a modification of a specific *o*-toluidine method (Hyvärinen & Nikkilä, 1962) or by a glucose oxidase technique.

2-deoxy-D-glucose ($\alpha + \beta$) and 3-*O*-methyl-α-D-glucopyranose were purchased from Koch Light Laboratories. Commercially available soluble insulin (Wellcome, 20 u./ml.) was used.

RESULTS

In preliminary experiments on seventeen rats injections of 2-deoxy-D-glucose (0·25 mg in 5 μl.), to a total number of more than 100, were systemically made throughout the hypothalamus. In the majority of instances no increase in gastric acid output ensued. To check that these animals were still capable of responding to an appropriate stimulus at the end of the experiment a systemic infusion of 3-*O*-methylglucose (which is a potent stimulus to vagally mediated gastric acid secretion in rats as

prepared in these experiments) was given, and an entirely normal acid secretory response occurred.

In a minority of cases, however, injection of 2-deoxy-D-glucose led to an increase in gastric acid output. All such successful injections were made into one part of the hypothalamus. It was established that the sensitive tissue was situated about 2 mm from the mid line, 2 mm posterior to the bregma and 1·5 mm below the horizontal zero plane on the system of co-ordinates used. A single injection of 2-deoxy-D-glucose (0·25 mg) into this region, on one side only, could evoke a secretion of acid from the

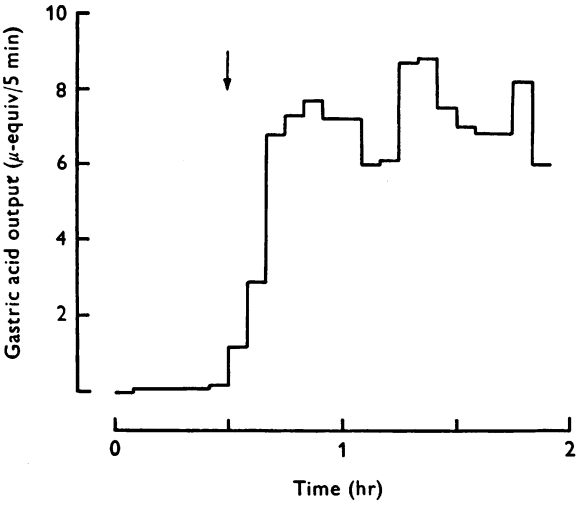

Fig. 1. The effect of a single intrahypothalamic injection of 2-deoxy-D-glucose (at arrow, 0·25 mg in 5 μl.) upon the gastric acid output.

stomach which was apparent within 5 min, rose to a maximum rate similar to that caused by hypoglycaemia or by the intravenous administration of 2-deoxy-D-glucose, and persisted over a long period of time (Fig. 1). The dose of 2-deoxy-D-glucose used in these experiments was approximately one two hundredth of that which will reliably cause the same effect when given by intravenous injection (50 mg).

Injections of 2-deoxy-D-glucose were made into this area in forty-one rats. In seventeen a marked rise in the gastric acid output occurred. In twenty-four no response was elicited. In eleven of these twenty-four animals, 2-deoxy-D-glucose was injected into the sensitive area on both sides without effect but only six of these secreted acid after the intravenous infusion of 3-*O*-methylglucose. In five, therefore, the mechanism controlling acid secretion under such circumstances had been inactivated. In the other thirteen rats out of the twenty-four which failed to respond

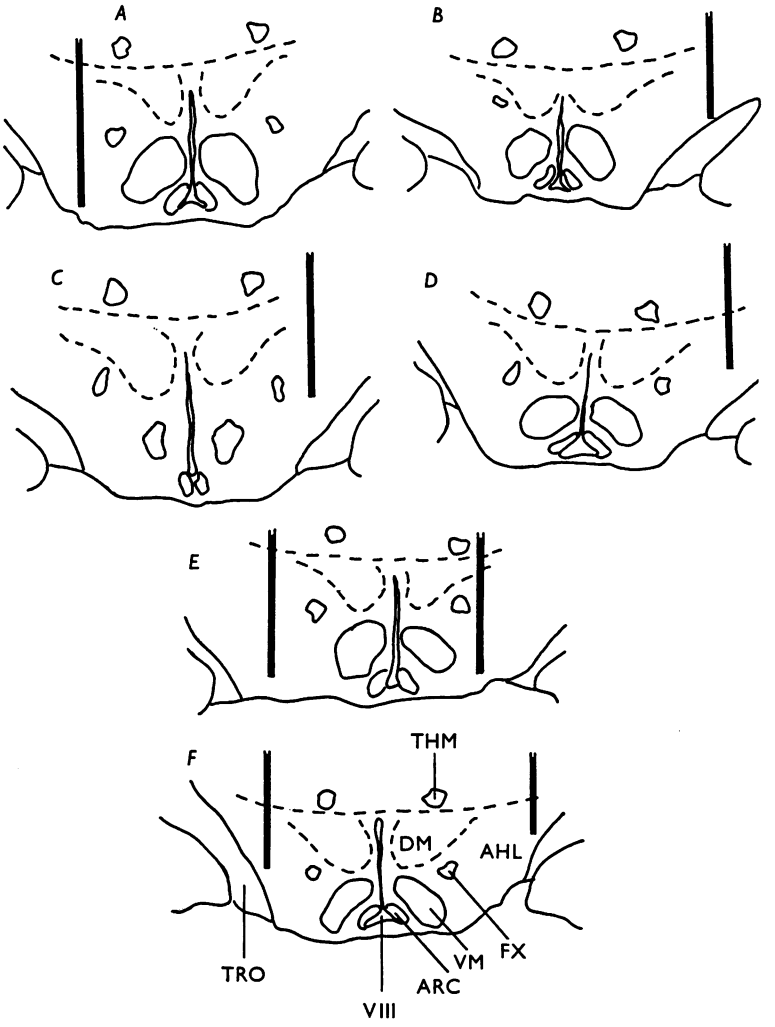

Fig. 2. A series of camera lucida drawings of typical coronal sections through the hypothalamus. The tracks of the cannulae are shown by the vertical black bars. *A*, *B*, *C* and *D* are from experiments where a single injection of 2-deoxy-D-glucose caused an increase in gastric acid output. (Sections *B* and D are from the rats in the experiments shown in Figs. 1 and 3 respectively.) *E* is from an experiment where gastric acid secretion, resulting from the intravenous infusion of 3-*O*-methylglucose, was ended by the injection of lignocaine into the hypothalamus on each side. *F* is from the rat in the experiment illustrated in Fig. 6*B*. AHL, lateral hypothalamic area; ARC, arcuate nucleus; DM, dorsomedial nucleus; FX, fornix; THM, mamillo thalamic tract; TRO, optic tract; VM, ventromedial nucleus; VIII, third ventricle.

injections were either made into one side of the hypothalamus only, in which case there was invariably normal secretory response to 3-*O*-methylglucose (showing that at least the centre of the other side was intact); or if bilateral injections were made the rats were not subsequently tested with 3-*O*-methylglucose.

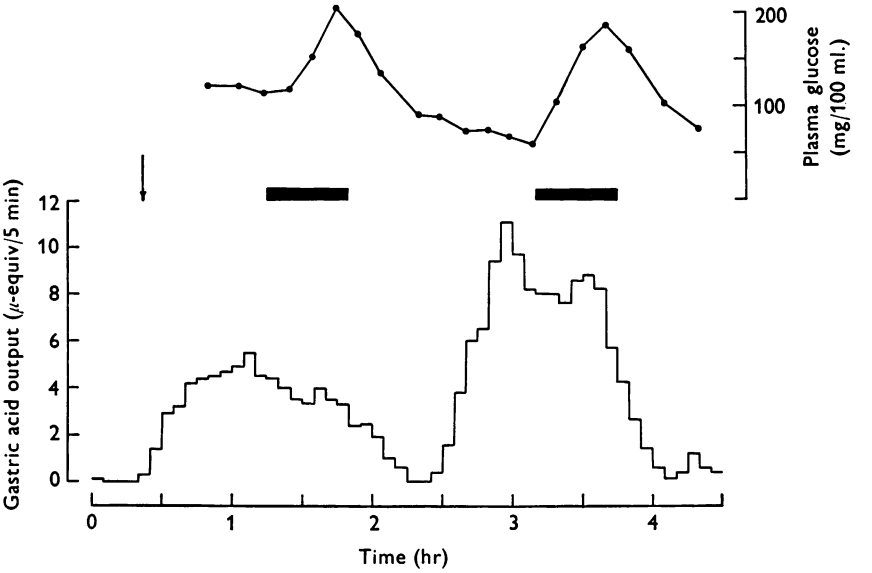

Fig. 3. The effect of the intravenous infusion of glucose (filled bars, 4 mg/min) upon the gastric acid output, caused by a single injection of 2-deoxy-D-glucose (at arrow, 0·25 mg) into the lateral hypothalamic area, and the plasma glucose concentration. Further details are given in the text.

Camera lucida drawings of typical coronal sections through the brains of some of the rats which responded to single intrahypothalamic injections of 2-deoxy-D-glucose by secreting gastric acid are shown in Fig. 2. It will be seen that in every case the track of the cannula descends into the lateral hypothalamic area, in the vicinity of the medial forebrain bundle, lateral to the fornix and well clear of the ventromedial nucleus.

This centrally provoked secretion of acid from the stomach was demonstrated to be a consequence of the metabolic block caused by 2-deoxy-D-glucose because it could be ended by the systemic administration of glucose. In the experiment illustrated in Fig. 3, acid secretion resulted from a single injection of 2-deoxy-D-glucose (0·25 mg) into the lateral hypothalamic area. Once acid secretion was established an intravenous infusion of glucose was begun at a rate of 4 mg/min. This caused the plasma glucose concentration to rise. After half an hour, acid secretion

began to diminish and the glucose infusion was stopped. The gastric acid output ceased entirely 25 min later. Shortly thereafter the plasma glucose concentration which had also been declining, reached normal, at which point acid secretion recommenced. This renewed secretion too was terminated by a second infusion of glucose. On each occasion when the decrease in acid output first became apparent the plasma glucose concentration was about the same, 203 and 186 mg/100 ml.; in another similar experiment it was 980 mg/100 ml.

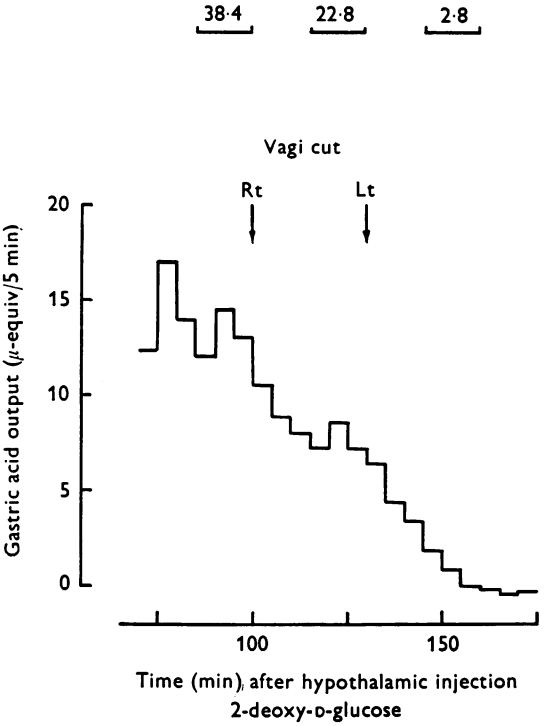

Fig. 4. The effect of cutting each vagus in turn upon the gastric acid secretion provoked by the injection of 2-deoxy-D-glucose into the lateral hypothalamic area on the right side. The quantity of acid secreted (μ-equiv.) during the 15 min periods before each nerve was cut and the corresponding period afterwards are also shown. Further details are given in the text.

Gastric acid secretion after the intrahypothalamic injection of 2-deoxy-D-glucose, like that due to hypoglycaemia, is dependent upon the vagi, because after the administration of atropine (0·5 mg, I.V.) or after bilateral cervical vagotomy acid production invariably stopped within 25 min. Furthermore, the chemosensitive area on each side of the hypothalamus is connected to both the right and the left vagal nerves. Cutting the right

vagus of a rat in which acid secretion was due to the injection of 2-deoxy-D-glucose into the right side of the hypothalamus caused the rate of secretion to diminish. However, after 30 min it was still continuing steadily albeit reduced by about 40% (Fig. 4). Only after the left nerve also was divided did the output of acid cease. The same two-stage reduction in acid output was seen in another similar experiment when the nerves

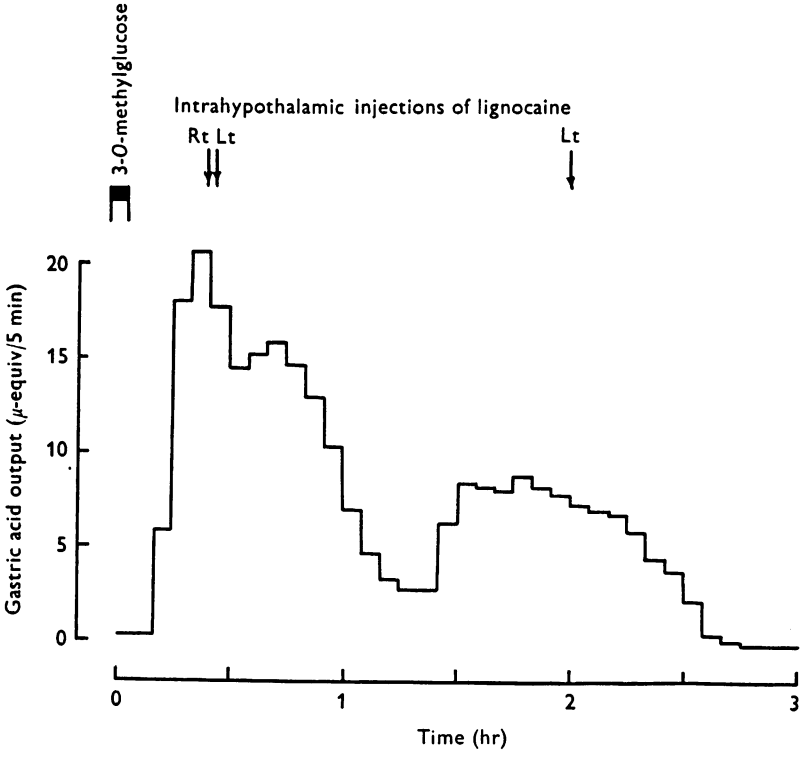

Fig. 5. The effect of injections of 3 μl. lignocaine into the lateral hypothalamic area on each side upon the gastric acid output caused by the intravenous infusion of 300 mg 3-*O*-methylglucose. During the course of the experiment a further 450 mg 3-*O*-methyglucose was given to maintain the plasma concentration of this substance. After the initial diminution, acid secretion increased suggesting some escape from the effects of the lignocaine. A single further injection of the same dose of lignocaine into the left side only finally abolished the gastric acid secretion.

were cut in the reverse order. In a parallel set of experiments in which gastric acid secretion was due to the intravenous infusion of 3-*O*-methylglucose, cutting one vagus in the neck caused a reduction in secretion to approximately 60% of the previous rate.

In the final series of experiments it was shown that the normal functioning of the lateral hypothalamic area is necessary if the gastric secretory response to a systemically delivered stimulus is to take place. The injection of a solution of lignocaine (3 μl.) into this region on each side brought to an end the secretion of acid resulting from the intravenous infusion of 3-*O*-methylglucose (Fig. 5). Injections of lignocaine made elsewhere in the hypothalamus did not have such an effect. Likewise the prior injection of phenol into the lateral hypothalamic area on each side prevented the rise in gastric acid output which occurs when the plasma glucose falls, under

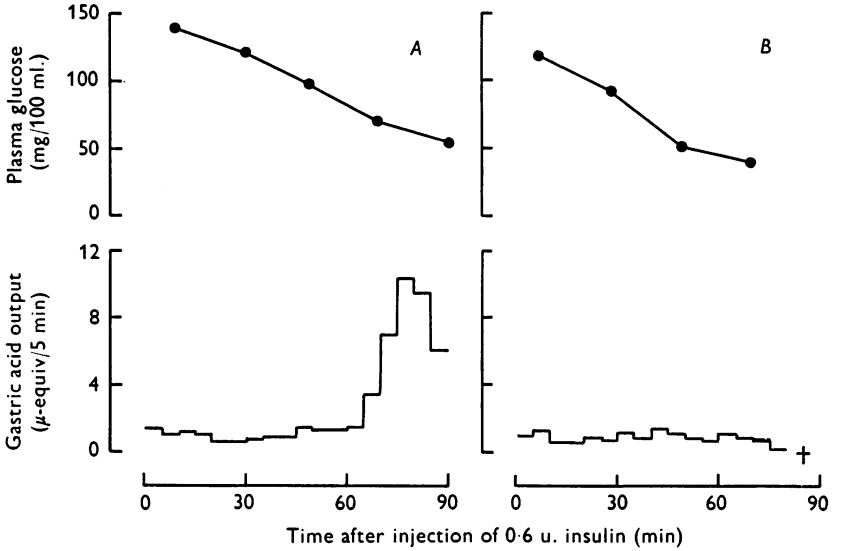

Fig. 6. The effect of bilateral injections of phenol into the lateral hypothalamic areas upon the gastric acid output provoked by subsequent insulin-induced hypoglycaemia. *A*, animal in which the response was unaffected by phenol injections (note that the threshold plasma glucose concentration for the onset of the response is normal (about 70 mg/100 ml.; see Colin-Jones & Himsworth, 1969)). *B*, animal in which injections of phenol prevented the secretions of acid during hypoglycaemia.

the influence of insulin, to below the critical level of 70 mg/100 ml. (Fig. 6). It should be noted that the injection of phenol into the lateral hypothalamic area did not itself cause any change in the negligible basal rate of gastric secretion.

DISCUSSION

The secretion of acid by the stomach may be provoked by hypoglycaemia following the injection of insulin or by the administration of either 3-*O*-methylglucose or 2-deoxy-D-glucose. Hypoglycaemia and 3-*O*-methyl-

glucose both cause a reduction in glucose oxidation by the central nervous system by diminishing the rate of transfer of glucose from the plasma into the cells of the brain (Himsworth, 1968). 2-deoxy-D-glucose also depresses the cerebral oxidation of glucose but from within the cell: the 6-phosphate ester of 2-deoxy-D-glucose acts as a potent, competitive, enzymic inhibitor of glycolysis (Tower, 1958). This property of 2-deoxy-D-glucose made it peculiarly suitable for use to create local and persistent changes in the glucose metabolism of different regions of the brain when attempting to find the position of the mechanism which determines the gastric secretory response to hypoglycaemia.

The injection, under stereotaxic control, of 0·25 mg 2-deoxy-D-glucose into the lateral hypothalamic area of rats on one side only was found to cause a marked and sustained increase in the gastric acid output in about half the experiments performed. The rate of acid secretion was similar to that evoked by insulin-induced hypoglycaemia and by the intravenous administration of 2-deoxy-D-glucose. The amount of 2-deoxy-D-glucose injected into the brain, however, was less than 1% of the minimum dose which will produce the same effect when given intravenously. The increase in gastric output cannot therefore be accounted for by absorption of 2-deoxy-D-glucose into the systemic circulation.

The fact that in twenty-four out of forty-one experiments intrahypothalamic injections of 2-deoxy-D-glucose produced no gastric response can be accounted for in some by the inactivation of the controlling mechanisms as shown by the subsequent failure to respond to systemically administered 3-*O*-methylglucose. This inactivation was probably due to a local overdose of 2-deoxy-D-glucose rather than to gross mechanical disruption because the latter was found in other experiments to cause an increase in acid output. This last observation is in accord with the finding by Ridley & Brooks (1965) of gastric hypersecretion immediately after bilateral electrolytic destruction of the ventromedial nuclei in rats. Discrete symmetrical lesions in the hypothalamus on each side of the mid line have been reported to abolish the gastric secretory response to hypoglycaemia in rats (Ridley & Brooks, 1965) and dogs (Davis & Weiner, 1969). However, the interpretation of the findings in these experiments is difficult because such lesions invariably caused a basal rate of gastric acid secretion as great as the maximal response provoked by hypoglycaemia in control animals. In some experiments injections of 2-deoxy-D-glucose were made into the sensitive region of the hypothalamus without causing a secretion of acid or interfering with the subsequent response to 3-*O*-methyl-glucose. These results suggest that the sensitive tissue occupied only a part of the gross area delimited and that in these experiments this had not been touched by the injection.

It is known that direct, electrical stimulation of the lateral hypothalamic region on one side in rats causes the basal rate of acid secretion by the stomach to increase and that this can be prevented by cutting both vagal nerves (Misher & Brooks, 1966). The increase in gastric acid secretion in the present experiments was not, however, a consequence of non-specific stimulation of this region: first, because the mechanical irritation caused by introducing the cannula did not affect the negligible basal output of acid; and secondly, and most significantly, because the secretion of acid in the present experiments was clearly correlated with alterations in glucose metabolism, being suppressed by an increase and then released by a subsequent decrease in the plasma glucose concentration (Fig. 3).

Inhibition of glucose metabolism by the injection of 2-deoxy-D-glucose into the lateral hypothalamic area on one side only is therefore a powerful and specific stimulus to acid secretion by the stomach and is good evidence for the existence of a controlling chemoreceptor in this region. The localization of the chemoreceptor depends, however, upon the assumption that 2-deoxy-D-glucose when injected under the conditions of these experiments does not become widely dispersed throughout the substance of the brain. There are a number of reasons for believing this to be so. First, the immediate onset of acid secretion after the injection had been made, which was seen in the majority of successful experiments, suggests that the sensitive elements were very close to the tip of the cannula. Secondly, injections made elsewhere in the hypothalamus were without effect upon the acid output, showing that there was no significant diffusion of 2-deoxy-D-glucose through the brain. And third, it has been shown that after hypothalamic injections, made under comparable circumstances, the injected material remains within a sphere, centred on the tip of the cannula and of a radius less than 0·5 mm (Wagner & de Groot, 1963). It seems probable therefore that the 2-deoxy-D-glucose is taken up by the chemoreceptor cells amongst others in the lateral hypothalamic area and remains within them; otherwise it would not be possible to explain why acid secretion persists for some hours after injection and, if it is inhibited by raising the plasma glucose concentration, recurs following the return of the plasma glucose to normal.

The secretion of acid by the stomach caused by the local application of 2-deoxy-D-glucose to the hypothalamus is mediated by the same peripheral nervous pathway as that caused by insulin hypoglycaemia or the systemic administration of 2-deoxy-D-glucose or 3-*O*-methylglucose. Moreover, the chemoreceptors on each side of the hypothalamus were shown to be connected to both vagi for cutting either nerve alone reduced but did not abolish the secretion due to the injection of 2-deoxy-D-glucose into one side of the hypothalamus. Further, as judged by the rate of gastric acid

production such unilateral stimulation could cause a maximal, vagally mediated response.

The injection of small amounts of lignocaine into the lateral hypothalamic area on each side, but not elsewhere was found to inhibit an established secretion of gastric acid resulting from the infusion of 3-*O*-methylglucose. Similarly placed injections of phenol prevented the rise in secretion which characteristically accompanies developing hypoglycaemia. The normal functioning of this part of the hypothalamus is therefore necessary if the response to a systemic stimulus is to occur and be maintained. These last experiments also provide evidence in support of the belief that insulin hypoglycaemia, 2-deoxy-D-glucose and 3-*O*-methylglucose all activate the same mechanisms to cause an increase in the gastric acid output. It is important to note that inactivation by phenol of that part of the hypothalamus which is concerned with the gastric response to hypoglycaemia does not result in a basal hypersecretion of acid. This is in marked contrast to the effect of electrolytic lesions of the hypothalamus which have been reported to interfere with the same response (*vide supra*; Ridley & Brooks, 1965; Davis & Weiner, 1969). Furthermore, the experiments in this paper on the inactivation of the chemoreceptors by lignocaine provide information about the central organization of the gastric response to hypoglycaemia. The absence of an increase in basal acid secretion under such circumstances indicates that these chemoreceptors do not exert an inhibitory influence upon a lower centre which is removed during hypoglycaemia. The failure of animals in which the receptors have been destroyed by phenol or anaesthetized with lignocaine to respond to a lack of metabolizable glucose, as a result of insulin-induced hypoglycaemia or systemic injection of 3-*O*-methylglucose, shows that the response of unanaesthetized receptors to these stimuli is a positive phenomenon depending upon the retention of function within the sensitive cells. Taking these two considerations together it would appear that we have in these receptors a tissue that responds by overactivity if its metabolism of carbohydrate is reduced below a critical level.

The five postulates for the definition and localization of a chemoreceptor within the central nervous system which are set out in the introduction to this paper have been fulfilled. 2-deoxy-D-glucose has been shown to be an effective stimulus to acid secretion by the stomach when given intravenously and when applied locally to one restricted region of the brain. The impulse to this secretion is conveyed by the same nervous pathway and can be ended by the intravenous infusion of glucose whichever method of administration is employed. Inactivation of the sensitive region prevents the response to a systemically delivered stimulus. It is justifiable therefore to speak of a chemoreceptor, located in the lateral hypothalamic

area, which responds to a lack of metabolizable glucose and which can initiate and sustain the vagally mediated secretion of acid by the stomach.

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