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The Effect of Nickel on Secretory Systems

STUDIES ON THE RELEASE OF AMYLASE, INSULIN AND GROWTH HORMONE

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The effects of Ni^{2+} on the release of amylase from rat parotids, insulin from mouse pancreatic islets and growth hormone from bovine pituitary slices were investigated. In all these secretory systems, Ni^{2+} was shown to inhibit release evoked by a variety of stimuli both physiological and pharmacological. Measurements of rates of substrate oxidation and tissue concentrations of ATP and 3′:5′-cyclic AMP suggest that this inhibitory action of Ni^{2+} does not arise through an effect on energy metabolism or cyclic AMP metabolism. It is concluded that although some effects of Ni^{2+} may involve antagonism between Ni^{2+} and Ca^{2+} in stimulus–secretion coupling, others appear to be independent of Ca^{2+}. It is suggested that Ni^{2+} may block exocytosis by interfering with either secretory-granule migration or membrane fusion and microvillus formation. The possible mode of action of Ni^{2+} and its potential use as a tool in the study of exocytosis are discussed.

In view of the primary role ascribed to Ca^{2+} in stimulus–secretion coupling (Douglas, 1968) and the contribution made by specific inhibitors in clarifying biochemical pathways, an inhibitor which interferes with the action of Ca^{2+} in initiating exocytosis could provide insight into the mechanism of secretion. The bivalent cations Ni^{2+} and Co^{2+} are known to inhibit contraction of cardiac muscle (Kaufman & Fleckenstein, 1965) and the effect of these ions can be reversed by doubling the extracellular concentration of Ca^{2+} (Kleinfeld & Stein, 1968). This suggests that these ions interfere with the uptake of Ca^{2+} and/or its action on excitation–contraction coupling. Evidence has also been obtained that presynaptic neurotransmitter release is inhibited by Ni^{2+} and Zn^{2+} (Benoit & Mambrini, 1970) and by Mn^{2+} (Meiri & Rahaminoff, 1972) and again that inhibition by these ions is due to competition with Ca^{2+}. The similarity between the effects of these bivalent cations on excitation–contraction coupling in heart muscle and on excitation–secretion coupling in presynaptic nerve terminals suggests that they act on a fundamental process common to both contraction and secretion. If this is so, bivalent cations should also inhibit secretion of protein hormones, and, if that inhibition is at a fundamental level, stimulation by secretagogues acting in different ways should be inhibited.

In this paper we report effects of Ni^{2+} on the release of amylase from rat parotids, insulin from mouse pancreatic islets and growth hormone from ox pituitary slices in reponse to a range of physiological and pharmacological stimuli. We also report the effects of Ni^{2+} on some metabolic parameters in mouse islets and ox pituitary slices. The data suggest that Ni^{2+} is a specific and potent inhibitor of release in all three systems at a fundamental level.

Experimental

Reagents

[U-^{14}C]Glucose and DL-β-hydroxy[3-^{14}C]butyrate were from The Radiochemical Centre, Amersham, Bucks., U.K. Prostaglandin E_2 was a gift from Dr. J. R. Pike of Upjohn Ltd., Kalamazoo, Mich., U.S.A. All other chemicals were from British Drug Houses Ltd., Poole, Dorset, U.K.

Methods

Assay of protein release. Parotid glands were obtained from male albino rats starved overnight and were cut into pieces weighing 8–15 mg. Two or three fragments were placed in a perifusion chamber and perifused with bicarbonate-buffered salt solution (Krebs & Henseleit, 1932) as described by Robberlecht

& Christophe (1971). The activity of amylase released into the medium was measured by the method of Bernfeld (1955).

Islets of Langerhans were isolated by a collagenase procedure (Coll-Garcia & Gill, 1969) from 3–4-week-old male mice starved overnight. Islets were incubated in bicarbonate medium either in batches of five islets (Ashcroft *et al.*, 1973) or as a batch of 40 islets in the perifusion apparatus described by Cooper *et al.* (1973) by using a peristaltic pump to obtain flow rates of approx. 0.2 ml/min. Insulin concentrations in incubation media or perifusates were measured by double-antibody radioimmunoassay (Coll-Garcia & Gill, 1969).

Bovine pituitary slices (approx. 0.3 mm) were obtained from heifers within 5 min of death, sliced with a hand microtome and incubated in bicarbonate medium as described by Schofield (1967). The concentration of growth hormone in the media was measured by a double-antibody radioimmunoassay (Schofield, 1967). To combine results of several experiments, release in each experiment was expressed as a percentage of release by control slices in the same experiment.

Measurement of metabolic parameters. The rate of oxidation of [U-^{14}C]glucose by mouse islets was measured as described by Ashcroft *et al.* (1973) and rates of oxidation of [U-^{14}C]glucose and DL-β-hydroxy[3-^{14}C]butyrate by bovine pituitary slices as described by McPherson & Schofield (1972).

The ATP content of mouse islets was measured by the firefly-luciferase assay (Ashcroft *et al.*, 1973). The ATP and cyclic AMP contents of pituitary slices were measured as described by Cooper *et al.* (1972).

Results

Release of amylase from parotid slices

The effect of Ni^{2+} on the stimulation of amylase release by adrenaline, *p*-chloromercuribenzoate and high K^+ is shown in Fig. 1. Fig. 1(*a*) shows that Ni^{2+} (2 mM) had no effect on basal release but blocked the release in response to adrenaline (10 μM). However, this effect was reversible, since subsequent removal of the Ni^{2+} permitted adrenaline stimulation of release to occur. Fig. 1(*b*) shows that 0.2 mM-Ni^{2+} also blocked the stimulation by adrenaline and that raising the extracellular concentration of Ca^{2+} from 1.3 to 5.2 mM overcame this inhibition. However, Ni^{2+} (0.5 mM) did not block stimulation of release by 72 mM-K^+ (Fig. 1*c*) nor *p*-chloromercuribenzoate (0.1 mM) (Fig. 1*d*).

Ni^{2+} inhibits the activity of amylase (about 20% inhibition at 0.5 mM-Ni^{2+}) and this effect was taken into account in the calculation of amylase activity in the medium.

Release of insulin from islets of Langerhans

Table 1 shows that Ni^{2+} (2 mM) almost completely inhibited insulin release evoked by glucose (16.7 mM) in the presence or absence of caffeine, by *p*-chloromercuribenzoate (0.2 mM) in the presence of 3.3 mM-glucose, and by tolbutamide (0.2 mg/ml), high K^+ concentration, or leucine (5 mM) all in the presence of 3.3 mM-glucose and 5 mM-caffeine. The rapidity of onset of inhibition of Ni^{2+} is shown in Fig. 2(*a*); addition of 2 mM-Ni^{2+} to islets perifused with 16.7 mM-glucose and 5 mM-caffeine returned the rate of insulin release to control values without any appreciable delay. The inhibition of glucose-stimulated insulin release by 2 mM-Ni^{2+} was at least partially reversible, since after a 9 min exposure to Ni^{2+} islets consequently responded to high glucose with increased insulin release (Fig. 2*b*). The effect of increasing Ni^{2+} concentrations on glucose-stimulated release is shown in Fig. 2(*c*). Some inhibition was detected with 0.05 mM-Ni^{2+} and the inhibition was complete at 0.5 mM-Ni^{2+}. The radioimmunoassay of insulin was not affected by Ni^{2+}.

Release of growth hormone from ox pituitary slices

Table 2 shows the effect of Ni^{2+} (0.2–1 mM) on the stimulation of growth-hormone release by Ba^{2+} (2.3 and 6.9 mM). Ni^{2+} did not alter basal growth-hormone release at 0.2 or 0.5 mM, although a small but significant ($p < 0.05$ by Student's *t* test) increase was seen at 1 mM. However, Ni^{2+} markedly inhibited Ba^{2+}-stimulated release at 0.2 mM and had a greater inhibitory effect at higher concentrations. There was no evidence that the higher Ba^{2+} concentration decreased the sensitivity to Ni^{2+}.

Ni^{2+} also markedly inhibited the stimulation of growth-hormone release by 20 μM *p*-chloromercuribenzoate (Table 3). To test the possibility that Ni^{2+} inhibits by competing with Ca^{2+}, the effect on *p*-chloromercuribenzoate-stimulated release was tested at 2.54 and 0.125 mM-Ca^{2+}. At the lower Ca^{2+} concentration the ability of *p*-chloromercuribenzoate to increase release was not impaired, confirming the Ca^{2+}-independence of *p*-chloromercuribenzoate stimulations observed earlier (Schofield, 1971). Moreover the sensitivity of the pituitary to Ni^{2+} was not altered by changes in the Ca^{2+} concentrations, suggesting that inhibition is independent of Ca^{2+}.

Ni^{2+} also inhibited K^+-induced growth-hormone release, and the dependence of this inhibition on Ca^{2+} was tested (Table 4). The stimulation by K^+ was not increased when the Ca^{2+} concentration was raised to 10 mM but was markedly decreased when the Ca^{2+} concentration was lowered to 0.125 mM, which confirms the Ca^{2+}-dependence of K^+ stimulation noted by Schofield & Cole (1971). At 10 mM-Ca^{2+} there was no evidence that the pituitary was protected against Ni^{2+} inhibition. However, there was some

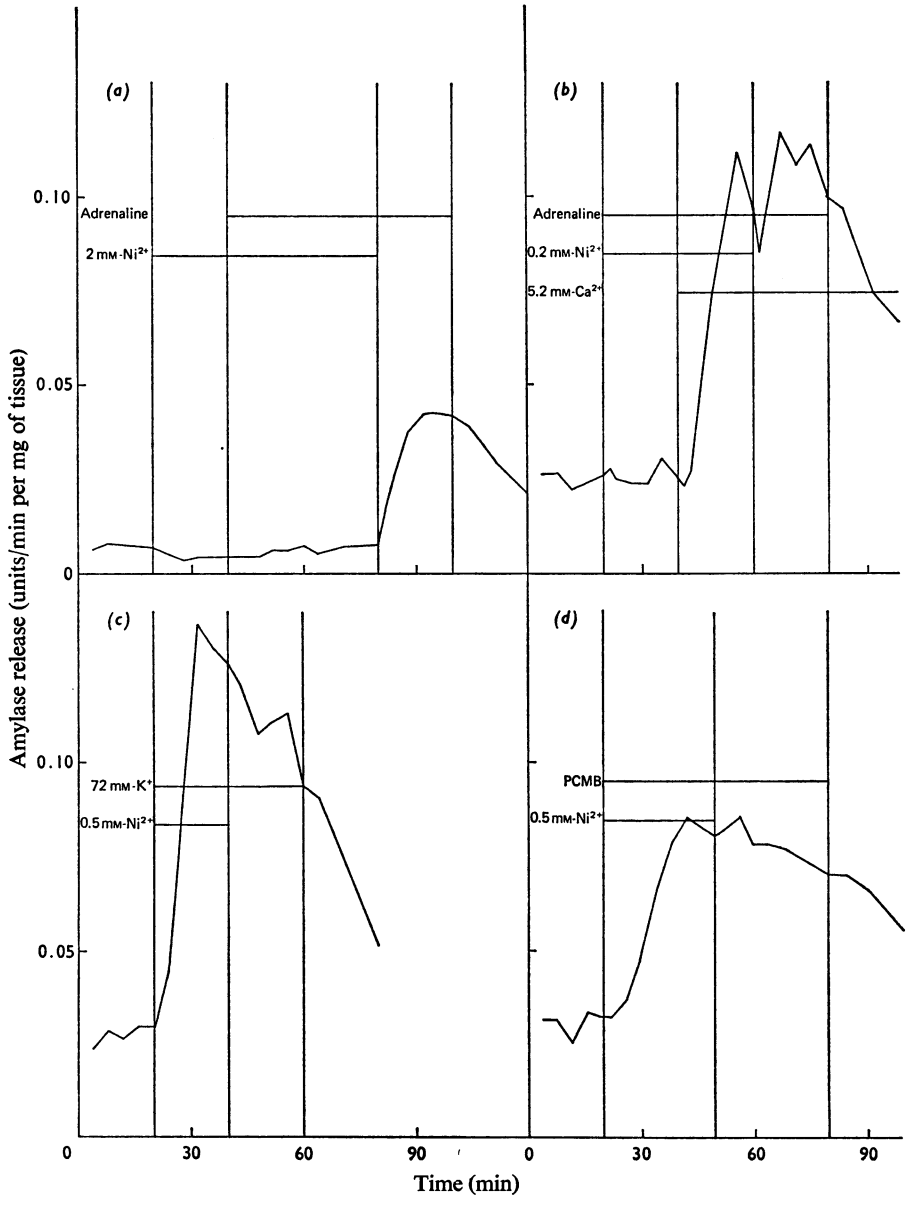

Fig. 1. *Effects of Ni^{2+} on amylase release from perifused parotid*

Parotid glands were perifused as described in the text with Krebs bicarbonate medium containing 5mM-β-hydroxybutyrate and other additions as follows. (*a*) Inhibition of adrenaline-stimulated release. 0–20min, no addition; 20–40min, 2mM-Ni^{2+}; 40–80min, 7mM-Ni^{2+}+10μM-adrenaline; 80–100min, 10μM-adrenaline; 100–130min, no addition. (*b*) Effect of Ca^{2+} on inhibition of adrenaline-stimulated release. 0–20min, 1.3mM-Ca^{2+}; 20–40min, 1.3mM-Ca^{2+}+0.2mM-Ni^{2+}+10μM-adrenaline; 40–60min, 5.2mM-Ca^{2+}+0.2mM-Ni^{2+}+10μM-adrenaline; 60–80min, 5.2mM-Ca^{2+}+10μM-adrenaline; 80–100min, 5.2mM-Ca^{2+}. (*c*) Effect on K^+-stimulated release. 0–20min, no additions; 20–40min, 0.5mM-Ni^{2+}+72mM-K^+: 40–60min, 72mM-K^+; 60–80min, no additions. (*d*) Effect on *p*-chloromercuribenzoate-stimulated release. 0–20min, no additions; 20–50min, 0.05mM-Ni^{2+}+0.1mM-*p*-chloromercuribenzoate; 50–80min, 0.1mM-*p*-chloromercuribenzoate; 80–100min, no additions. Amylase in the perifusates was measured as described in the text. PCMB, *p*-chloromercuribenzoate.

Table 1. *Effects of Ni^{2+} on insulin release*

Batches of five islets were incubated for 2h in bicarbonate medium containing albumin (2mg/ml) at 37°C under the conditions given. Insulin released into the medium was assayed by radioimmunoassay. Results are given as mean±S.E.M. for the number of batches of islets given in parentheses.

Incubation conditions			Insulin release (pg/min per islet)	
Glucose concn. (mM)	Caffeine (5mM)	Other additions	No Ni^{2+}	2mM-Ni^{2+}
3.3	−	—	8.26± 2.33 (20)	3.67±1.37 (15)
16.7	−	—	31 ± 3.7 (10)	4.25±1.25 (5)
3.3	+	—	7.08± 1.02 (25)	4.63±1.87 (10)
16.7	+	—	263 ±36 (25)	16.3 ±8.77 (20)
3.3	−	*p*-Chloromercuribenzoate (0.2mM)	282 ±40 (5)	<0.25 (5)
3.3	+	Tolbutamide (0.2mg/ml)	72 ± 5 (5)	<0.25 (5)
3.3	+	K^+ (60mM)	31.5 ± 0.4 (5)	0.4 ±0.2 (5)
3.3	+	Leucine (5mM)	126 ±13 (5)	2.7 ±1.5 (5)

evidence that at 0.12mM-Ca^{2+} the sensitivity to Ni^{2+} was increased. Thus the K^+-induced release was abolished by 0.2mM-Ni^{2+} in the presence of 0.12mM-Ca^{2+} but only halved in the presence of 2.5mM-Ca^{2+}. It was also observed that 10mM-Ca^{2+} caused a decrease in basal release to 73±6% of the control value.

The results of experiments to test the reversibility of Ni^{2+} inhibition are shown in Table 5. Slices incubated in high K^+ responded after a 30min lag period to a second high-K^+ stimulation with almost the same output of growth hormone as during the first high-K^+ period. Slices incubated in Ni^{2+} and high K^+ and then given a 30min rest period also responded to high K^+ in the absence of Ni^{2+} in the second stimulation period although the reversal was not complete.

Prostaglandin-E_2-induced release of growth hormone was also markedly inhibited by Ni^{2+}. Slices incubated for 60min with prostaglandin E_2 (1 μM) released 1.66±0.23mg of growth hormone/h per g of tissue compared with control release rate of 0.72±0.06mg/h per g. In the presence of 1mM-Ni^{2+} and prostaglandin E_2 the release rate was 0.72±0.15 mg/h per g (all values are mean±S.E.M. for 16 slices).

Effects of Ni^{2+} on metabolism

The results given in Table 6 show that for mouse islets incubated for 2h *in vitro* neither the ATP content nor the rate of formation of $^{14}CO_2$ from [U-^{14}C]glucose was significantly affected by the presence of 2mM-Ni^{2+} ($p<0.05$ by Student's *t*-test).

Ni^{2+} (0.5mM) did not significantly decrease the rate of oxidation of glucose or of β-hydroxybutyrate (Table 7) by ox pituitary slices over a 60min period. Ni^{2+} (2mM) decreased the rate of oxidation of glucose but not of β-hydroxybutyrate under these conditions. However, preincubation for 90min in Ni^{2+} (0.5 or 2mM) decreased the rates of oxidation of both glucose and β-hydroxybutyrate in a subsequent 60min incubation in the presence of Ni^{2+}. No changes in pituitary content of ATP content were observed in slices incubated with 2mM-Ni^{2+}. The basal concentration of cyclic AMP was not altered by 1mM-Ni^{2+}, nor was the ability of prostaglandin E_2 to raise the concentration of cyclic AMP (suggesting no change in adenylate cyclase activity), nor was the increase in cyclic AMP elicited by the inhibitor of phosphodiesterase, 3-isobutylmethylxanthine (suggesting that phosphodiesterase activity was also unaffected).

Discussion

The present study demonstrates that Ni^{2+} is a potent inhibitor of secretion in three glands, parotid (amylase), islets of Langerhans (insulin) and pituitary (growth hormone). These findings are at variance with the recent report of La Bella *et al.* (1973) that Ni^{2+} at the concentration used here stimulated release of a number of hormones from bovine pituitaries *in vitro*. We have observed no stimulation of growth-hormone release from bovine pituitaries by Ni^{2+}. We suggest that the long delay (60min) between the death of the animal and removal of the pituitary in the experiments of La Bella *et al.* (1973) could drastically change the behaviour of the pituitary *in vitro* and thus account for this discrepancy.

The secretory process in parotid, islet β-cells and pituitary is thought to involve exocytosis, that is,

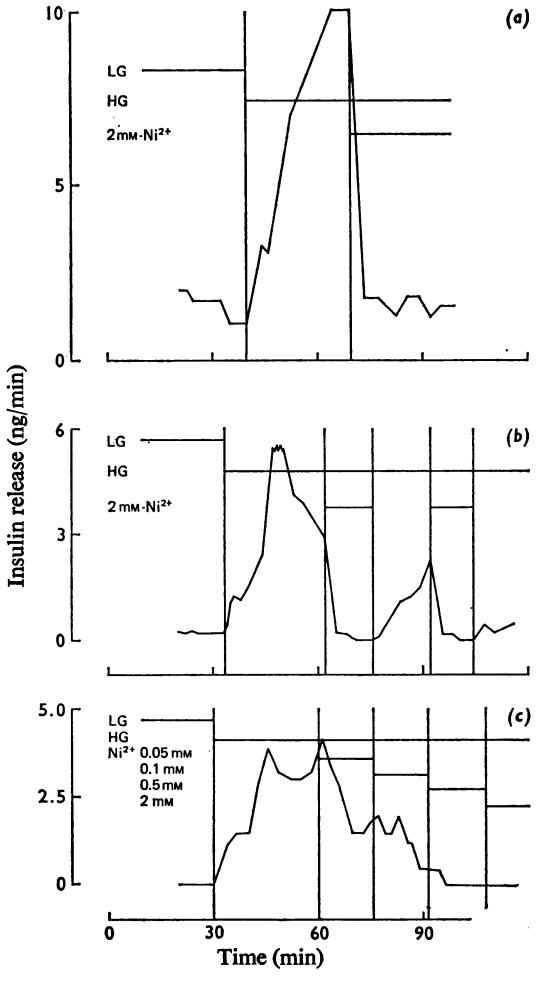

Fig. 2. *Effects of Ni^{2+} on insulin release from perifused mouse islets*

Batches of 40 islets were perifused as described in the text with Krebs bicarbonate medium containing albumin (2mg/ml) and caffeine (1mg/ml) and the following additions. (*a*) 0–40 min, 3.3mM-glucose (LG); 40–70min, 16.7mM-glucose (HG); 70–100min, HG+2mM Ni^{2+}. (*b*) 0–33min, LG; 33–64min, HG; 64–76min, HG+2mM Ni^{2+}; 76–92min, HG; 92–104min, HG+2mM-Ni^{2+}; 104–116min, HG. (*c*) 0–30min, LG; 30–60min, HG; 60–76min, HG+0.05mM-Ni^{2+}; 76–92min, HG+0.1mM-Ni^{2+}; 92–108min, HG+0.5mM-Ni^{2+}; 108–120min, HG+2mM-Ni^{2+}. Insulin in the perifusates was measured by radioimmunoassay as described in the text.

migration of secretory granules to the plasma membrane followed by discharge of granule contents through the plasma membrane (Amsterdam *et al.*, 1969; Lacy, 1961; Farquhar, 1961). Stimulation of secretion by physiological agents and many drugs is dependent on extracellular Ca^{2+} (Selinger & Naim, 1970; Milner & Hales, 1968; Schofield, 1971) and on adequate intracellular concentrations of ATP (Babad *et al.*, 1967; Ashcroft *et al.*, 1973; Schofield & Stead, 1971). It is therefore suggested that stimulus–secretion coupling is mediated by uptake of extracellular Ca^{2+} and/or by release of Ca^{2+} from intracellular organelles and that ATP is utilized in exocytosis. Cyclic AMP may also augment the secretory process by an unknown mechanism because agents that increase intracellular cyclic AMP accelerate secretion in the three tissues that we have studied (Malamud, 1972; Cooper *et al.*, 1972, 1973). Thus possible sites for the inhibitory action of Ni^{2+} are ATP synthesis, cyclic AMP metabolism and Ca^{2+} uptake and/or action.

In isolated islets and in isolated pituitary slices Ni^{2+} inhibited secretory responses to agents which depend for their effect on extracellular Ca^{2+} [glucose, leucine, tolbutamide and high K^+ in islets; high K^+ and prostaglandin E_1 in pituitary slices (Milner & Hales, 1968; Cooper *et al.*, 1972)]. Ni^{2+} blocked secretory responses which may not involve increasing the intracellular cyclic AMP [e.g. glucose in islets (Montague & Cook, 1971; Cooper *et al.*, 1973)] and it also blocked secretory responses associated with increased cyclic AMP, e.g. methylxanthine potentiation in islets (Montague & Cook, 1971; Cooper *et al.*, 1972), adrenaline in parotid (Malamud, 1972) and prostaglandin E_1 in pituitary (Cooper *et al.*, 1972).

These effects of Ni^{2+} do not appear to result from interference with energy metabolism and ATP synthesis. Thus in islets Ni^{2+} did not change the rate of glucose oxidation or islet content of ATP. In pituitary slices, Ni^{2+} inhibited oxidation of glucose and 3-hydroxybutyrate but it did not lower ATP concentration, and the inhibitory effects on oxidation required higher concentrations of Ni^{2+} or longer exposure to Ni^{2+} than was necessary to give inhibitory effects on release. No evidence has been obtained that Ni^{2+} interferes with the metabolism of cyclic AMP; thus in pituitary slices Ni^{2+} did not lower either the basal cyclic AMP concentration or the elevated concentration after exposure to prostaglandin E_1. This suggests that Ni^{2+} may act by inhibiting some aspect of stimulus–secretion coupling which is fundamental to the action of a number of different types of secretagogue and regardless of their mechanism.

Some evidence for antagonism between Ca^{2+} and Ni^{2+} was obtained with adrenaline stimulation of amylase release, where the Ni^{2+} effect was partially reversed by raising the Ca^{2+} concentration, and with high K^+ stimulation in the pituitary, where sensitivity to inhibition by Ni^{2+} was increased by lowering extracellular Ca^{2+} concentration. The ability of Ni^{2+} to inhibit stimulation of growth-hormone release by Ba^{2+} could also be interpreted as indirect evidence

Table 2. *Effect of Ni^{2+} on the stimulation of growth hormone release by Ba^{2+}*

In each experiment three or four bovine pituitary slices were incubated at 37°C in 2ml of Krebs bicarbonate medium (containing 2.5mM-glucose, 2.5mM-sodium glutamate and 2.5mM-β-hydroxybutyrate) in which SO_4^{2-} was replaced by Cl^-. Ba^{2+} and Ni^{2+} were present at the concentration given. After incubation (60min) the tissue was removed from the medium, blotted and weighed and the growth hormone released into the medium was determined by radioimmunoassay. Release from each slice was expressed as a percentage of the mean rate of release by four control (i.e. no Ni^{2+} or Ba^{2+}) slices in the same experiment, and the data in the table are mean values ± S.E.M. for several incubations under each condition; the numbers of slices are given in parentheses.

	Growth-hormone release (% of control)		
Ni^{2+} concn. (mM)	No Ba^{2+}	2.3mM-Ba^{2+}	6.9mM-Ba^{2+}
0	100± 3 (137)	260±17 (30)	511±52 (29)
0.2	139±24 (25)	185±19 (17)	363±31 (16)
0.5	92±10 (27)	141±28 (28)	153±23 (12)
1.0	124± 9 (36)	111±23 (4)	—

Table 3. *Effect of Ni^{2+} on the stimulation of growth-hormone release by p-chloromercuribenzoate at two Ca^{2+} concentrations*

In each experiment four slices were incubated in the presence or absence of 20μM-*p*-chloromercuribenzoate with the concentrations of Ni^{2+} and Ca^{2+} given in the table. The data given are mean values ± S.E.M. for the rates of release under each condition expressed as a percentage of the rate of release by four control slices from the same pituitary incubated at 2.54mM-Ca^{2+} in the absence of *p*-chloromercuribenzoate with the total number of slices under each condition given in parentheses. For other details see the legend to Table 2.

	Growth-hormone release	
Ni^{2+} concn. (mM)	2.54mM-Ca^{2+}	0.125mM-Ca^{2+}
0	844±92 (24)	890±106 (16)
0.2	618±123 (8)	591±76 (8)
0.4	554±78 (12)	—
1.0	307±42 (16)	313±85 (8)

for antagonism between Ni^{2+} and Ca^{2+}. In both the adrenal medulla (Douglas, 1968) and the pancreatic β-cell (Milner & Hales, 1968) the effect of Ba^{2+} is independent of Ca^{2+}, and it is assumed that it either replaces Ca^{2+} in the secretory process or displaces Ca^{2+} from intracellular binding sites. However, the stimulation of growth hormone release by *p*-chloromercuribenzoate is not dependent on extracellular Ca^{2+} (Schofield, 1971). Nevertheless, this stimulation was substantially inhibited by Ni^{2+}. This would suggest that Ni^{2+} may have direct effects on the process of exocytosis which are distinct from any effects that it may have on Ca^{2+} uptake or action. The nature of this postulated direct effect of Ni^{2+} is not known, but likely sites are granule migration or membrane fusion and microvillus formation. Electron microscopy may provide further evidence on this point. Ni^{2+} may thus prove to be a useful tool in identifying components of the secretory system and in characterizing their role in the secretory process.

Table 4. *Effect of Ni^{2+} on K^+-stimulated growth-hormone release at different Ca^{2+} concentrations*

In each experiment, four slices were incubated in medium in which the K^+ concentration was increased to 72mM and the Na^+ concentration correspondingly lowered. The data are presented as in Table 3.

	Growth-hormone release (% of control)		
Ni^{2+} concn. (mM)	10mM-Ca^{2+}	2.5mM-Ca^{2+}	0.12mM-Ca^{2+}
0	437±46 (20)	484±42 (60)	248±32 (16)
0.1	—	—	142±18 (8)
0.2	316±88 (8)	311±31 (20)	131±15 (12)
0.4	207±23 (12)	164±18 (12)	104±12 (12)
0.5	—	132±10 (12)	—
1.0	—	162±12 (24)	—

Table 5. *Reversibility of the inhibition by Ni^{2+} of K^+-stimulated growth-hormone release*

Slices were incubated in the presence or absence of Ni^{2+} (1 mM) in normal or 72 mM-K^+ media for 60 min (period 1). Slices were transferred to control medium for 30 min and then reincubated in normal or high-K^+ media (period 2). Growth-hormone release during periods 1 and 2 is given as means ± S.E.M. for twelve slices in three experiments.

Incubation conditions		Growth-hormone release (mg/h per g of wet tissue)	
K^+ concn.	Ni^{2+} (in period 1 only)	Period 1	Period 2
Normal	−	1.06 ± 0.14	0.71 ± 0.10
High (72 mM)	−	3.75 ± 0.38	3.91 ± 0.31
Normal	+	1.02 ± 0.11	0.93 ± 0.15
High (72 mM)	+	1.37 ± 0.16	2.11 ± 0.27

Table 6. *Effect of Ni^{2+} on the oxidation of [U-^{14}C] glucose and the content of ATP in mouse pancreatic islets*

For measurement of glucose oxidation, batches of ten islets were incubated for 2 h at 37°C in Krebs bicarbonate medium containing [U-^{14}C]glucose (3 mCi/mmol). Rates of glucose oxidation were determined as described in the text. For measurement of islet ATP content batches of six islets were incubated for 2 h at 37°C in Krebs bicarbonate medium containing albumin (2 mg/ml) and caffeine (5 mM). After incubation islets were extracted with $HClO_4$ and ATP was determined by a luciferase assay. Results are given as means ± S.E.M. with the numbers of batches of islets in parentheses.

Incubation conditions		Glucose oxidation	ATP content
Glucose concn. (mM)	Ni^{2+} concn. (mM)	(pmol/h per 10 islets)	(pmol/islet)
10	—	190 ± 13 (10)	—
10	0.5	160 ± 10 (10)	—
10	2	161 ± 13 (10)	—
3.3	—	—	9.7 ± 0.6 (5)
	2		11.3 ± 1.3 (5)
16.7	—	—	10.6 ± 1.8 (5)
	2	—	14.3 ± 1.4 (5)

Table 7. *Effects of Ni^{2+} on metabolic parameters in pituitary slices*

For measurement of oxidation rates, pituitary slices were either (a) incubated for 60 min at 37°C in Krebs bicarbonate medium containing the radioactive substrate and Ni^{2+} as described in the text or (b) preincubated for 90 min at 37°C with the non-radioactive substrate and Ni^{2+} and then incubated for a further 60 min at 37°C with radioactive substrate and Ni^{2+}. For other details see the text. Data represent mean ± S.E.M. for the numbers of observations given in parentheses.

		Ni^{2+} concn. (mM)				No. of observations
		0	0.5	1.0	2.0	
Glucose oxidation rate	(a)	2.45 ± 0.34	2.42 ± 0.36	—	1.27 ± 0.08	(8)
(pmol/h per g wet wt. of tissue)	(b)	0.81 ± 0.07	0.49 ± 0.04	—	0.36 ± 0.04	(16)
β-Hydroxybutyrate oxidation rate	(a)	2.16 ± 0.25	2.49 ± 0.16	—	1.84 ± 0.17	(8)
(μmol/h per g wet wt. of tissue)	(b)	1.28 ± 0.20	0.87 ± 0.14	—	0.42 ± 0.04	(8)
ATP content (nmol/mg wet wt. of tissue)		0.77 ± 0.03	—	0.82 ± 0.06	—	(8)
Cyclic AMP content (pmol/ g wet wt. of tissue)						
Basal		0.04 ± 0.01	—	0.05 ± 0.01	—	(8)
+ Prostaglandin E_2 (1 μM)		0.17 ± 0.04	—	0.24 ± 0.05	—	(8)
+ 3-Isobutylmethylxanthine (1 mM)		1.57 ± 0.22	—	1.34 ± 0.12	—	(8)

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References

Amsterdam, A., Ohad, I. & Schramm, M. (1969) *J. Cell Biol.* **41**, 753
Ashcroft, S. J. H., Weerasinghe, L. C. C. & Randle, P. J. (1973) *Biochem. J.* **132**, 223–231
Babad, H., Ben-Zui, R., Bdolah, A. & Schramm, M. (1967) *Eur. J. Biochem.* **1**, 96–101
Benoit, P. R. & Mambrini, J. (1970) *J. Physiol.* **210** 681–695
Bernfeld, P. (1955) *Methods Enzymol.* **1**, 149–158
Coll-Garcia, E. & Gill, J. R. (1969) *Diabetologia* **5**, 61-66
Cooper, R. H., McPherson, M. & Schofield, J. G. (1972) *Biochem. J.* **127**, 143–154
Cooper, R. H., Ashcroft, S. J. H. & Randle, P. J. (1973) *Biochem. J.* **134**, 599–605
Douglas, W. W. (1968) *Brit. J. Pharmacol.* **34**, 451–474
Farquhar, M. (1961) *Trans. N. Y. Acad. Sci.* **23**, 346–351
Kaufman, R. & Fleckenstein, A. (1965) *Pflügers Arch. Gesamte Physiol. Menschen Tiere* **282**, 290–297
Kleinfeld, M. & Stein, E. (1968) *Amer. J. Physiol.* **215**, 593–599
Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66.
La Bella, F., Dular, R., Vivian, S. & Queen, G. (1973) *Biochem. Biophys. Res. Commun.* **52**, 786–791
Lacy, P. E. (1961) *Amer. J. Med.* **31**, 851–859
Malamud, D. (1972) *Biochim. Biophys. Acta* **279**, 373–376
McPherson, M. & Schofield, J. G. (1972) *FEBS Lett.* **24**, 45–48
Meiri, U. & Rahaminoff, R. (1972) *Science* **176**, 308–309
Milner, R. D. G. & Hales, C. N. (1968) *Biochim. Biophys. Acta* **150**, 165–167
Montague, W. & Cook, J. R. (1971) *Biochem. J.* **122**, 115–120
Robberlecht, P. & Christophe, J. (1971) *Amer. J. Physiol.* **220**, 911–917
Schofield, J. G. (1967) *Biochem. J.* **103**, 331–341
Schofield, J. G. (1971) *Biochim. Biophys. Acta* **252**, 516–525
Schofield, J. G. & Cole, E. H. (1971) *Mem. Soc. Endocrinol.* **19**, 185–199
Schofield, J. G. & Stead, M. (1971) *FEBS Lett.* **13**, 149–151
Selinger, Z. & Naim, E. (1970) *Biochim. Biophys. Acta* **206**, 335–337