VIP enhances TRH-stimulated prolactin secretion of pituitary tumours

Studies with ^{31}P NMR

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Intravenous thyrotrophin releasing hormone (TRH) caused a 6.5-fold increase in plasma prolactin (PRL) in rats carrying implanted pituitary tumours. Vasoactive intestinal polypeptide (VIP) had no effect, but TRH given after VIP raised TRH stimulated secretion 13-fold above basal. ^{31}P NMR spectroscopy showed that VIP caused a decrease in high energy metabolites (depleted phosphocreatine, elevated inorganic phosphate and lowered intracellular pH). TRH alone caused a similar but smaller effect; given after VIP, it caused no detectable depletion. We suggest that the changes in high energy metabolite cencentrations reflect increased cellular energy consumption consistent with a priming process (stage 1) in PRL secretion, followed by hormone release (stage 2). VIP induces stage 1 whereas RTH induced both stages.

^{31}P NMR *Vasoactive intestinal polypeptide* *Thyrotrophin releasing hormone* *Prolactin* *Pituitary tumor*

1. INTRODUCTION

Stimulation of PRL secretion is less well understood than inhibition. TRH is known to increase secretion but it is likely that other factors also take part. Several authors have reported that VIP can increase PRL secretion [1–3], and VIP is present in high concentrations in the hypothalamus [4] and in pituitary portal blood of rats [5]. However, the effect of VIP varies with the experimental method used and there are conflicting views as to its importance.

Recent developments in nuclear magnetic resonance (NMR) spectroscopy using surface coil methods [6–8] have permitted investigation of metabolic changes in whole organs. This is particularly useful in studying highly labile phosphate compounds which may be degraded by conventional freeze-clamping and extraction but can be measured non-invasively by ^{31}P NMR.

We have previously shown [9] that PRL-secreting tumours arising spontaneously in rats provide a very good experimental model for studying the hormonal control mechanisms of human prolactinomas; they continue to function even when transplanted to subcutaneous sites. We have therefore applied ^{31}P NMR spectroscopy to these tumours in the living animal to study the intracellular metabolic changes accompanying stimulation of PRL secretion by TRH and VIP.

Abbreviations: TRH, thyrotrophin releasing hormone; PRL, prolactin; VIP, vasoactive intestinal polypeptide; PCR, phosphocreatine; P_i, inorganic phosphate

2. MATERIALS AND METHODS

2.1. *Hormone experiments*

Tissue originating from spontaneously arising PRL-secreting rat pituitary tumours was trans-

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planted subcutaneously into the flanks of young female Wistar rats as in [9]. When the resulting tumours were greater than 2 cm in diameter the animals were treated daily with 5 μg subcutaneous oestradiol benzoate for 3 days. On the fourth day they were anaesthetised with urethane (0.114 g/kg) and the external jugular vein was catheterised. After equilibration for 1 h, 3–5 blood samples were taken at intervals of 10 min. Rats were then given, as a bolus i.v. injection, either 8 μg/100 g VIP or 0.8 μg/100 g TRH at the times shown in fig.1.

PRL was measured using the protocol and materials supplied by NIADDK (Bethesda, MD).

2.2. *NMR*

After 3 days oestradiol treatment, rats bearing transplanted PRL-secreting tumours were anaesthetised and their jugular veins catheterised. A 2-turn, 2 cm diameter radiofrequency coil was placed on the skin over the tumour and the animal was placed within the bore (27 cm) of an Oxford Research Systems TMR-32 spectrometer. The surface coil had an external standard consisting of a capillary containing methylene diphosphonate mounted in its plane.

Experiments were performed using a sweep width of 2 kHz, 15 μs radiofrequency pulses and a 2 s recycle time. Studies with phantoms showed that these conditions gave signals almost entirely from the tumour and not from skin or underlying muscle. Control experiments with longer recycle times showed that signals were not lost by saturation.

The resonances were integrated by the routine supplied with the spectrometer after deconvolution to remove underlying broad components. Control experiments in which peaks were cut out from spectra and weighed, showed that deconvolution did not significantly alter relative peak integrals. The interspectral coefficient of variation of the integrals of the peaks of the methylene diphosphonate external standard was 6.3%. Intracellular pH was measured from the chemical shift of the P_i resonance [8].

3. RESULTS

3.1. *Effects of VIP and TRH on prolactin secretion*

The intravenous injection of TRH to tumour bearing rats was followed by the well recognized increase in plasma prolactin concentrations, up to 6.5-fold above the basal values of 419 ± 127 (SE) ng/ml (fig.1). In these tumours a single intravenous injection of VIP alone had no significant effect on prolactin secretion, but if TRH was given 40 min after VIP, prolactin secretion rose up to 13-fold above basal values (fig.1). In similar experiments with normal rats in which basal prolactin concentrations were 14.8 ± 2.7 ng/ml, VIP given in the same dosage produced a slight but non-significant effect on prolactin secretion, and after the subsequent injection of TRH the mean plasma hormone value was 32.4 ± 2.5 ng/ml.

3.2. *Effect of VIP and TRH on* ^{31}P *NMR spectra*

Typical NMR spectra obtained from a prolactin-secreting tumour without treatment, after the administration of VIP alone, and after TRH alone are shown in fig.2. In the control, untreated,

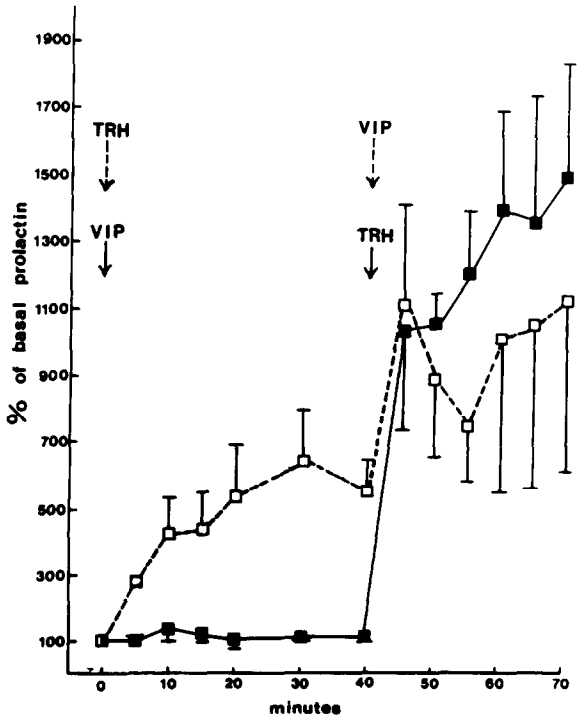

Fig.1. Effect of VIP and TRH on plasma prolactin concentrations in rats with transplanted prolactin-secreting pituitary tumours. Values are expressed as percentages of basal hormone secretion ± SE. Order of peptides given was: (■—■) VIP followed by TRH (n = 5); (□—□) TRH followed by VIP (n = 5).

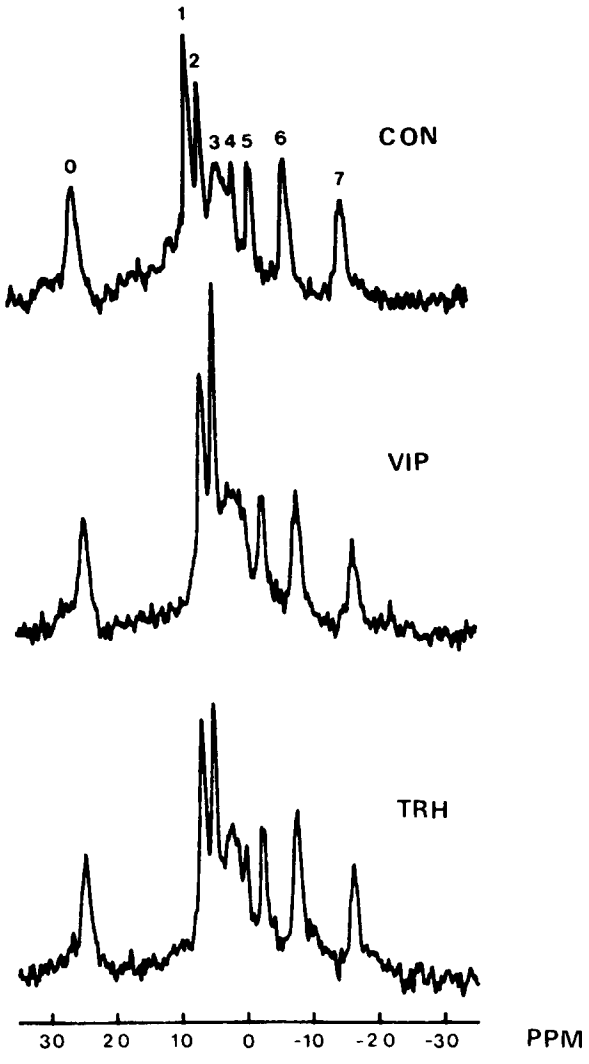

Fig.2. NMR spectra from a transplanted prolactin-secreting pituitary tumour in the living rat. CON, untreated; VIP, 4 min after injection; TRH, 4 min after injection. (0) Methylene diphosphonic acid added as external standard, (1) phosphomonoesters, (2) inorganic phosphate, (3) phosphodiesters, (4) phosphocreatine, (5) γATP + βATP, (6) αATP + ADP, (7) βATP.

tumour spectrum, 7 peaks were present comprising the α-phosphate of ATP (together with some NAD and ADP), the β-phosphate of ATP, the γ-phosphate of ATP (with some ADP), PCr, phosphodiesters, P_i and phosphomonoesters. Treatment with VIP produced a considerable increase in the P_i peak and a reduction in the PCr peak. These changes occurred within 4 min of giving the peptide and usually returned to the resting level after about 24 min. Changes observed after giving TRH were much smaller.

Fig.3 shows the mean quantitative effects after injections of VIP or TRH on the P_i content in 11 tumours. Both peptides increased P_i but the increase produced by VIP (60% above basal values) was significantly greater ($p<0.05$) than that produced by TRH (25% above basal values). In addition, VIP caused a significant fall in the intracellular pH of the tumours from 7.05 ± 0.06 to 6.7 ± 0.1 ($p<0.05$) which was not seen after TRH. The effect of TRH administered after VIP is shown in the right hand portion of fig.3. There were no significant changes in P_i or intracellular pH. On the other hand, when VIP was given after TRH it still induced a rise in P_i and a fall in the intracellular pH (not shown).

3.3. *Vasodilation by VIP*

It is well recognized that VIP has very potent vasodilator properties and to exclude the possibility that changes in blood flow could affect the NMR spectra, control experiments were carried out with intravenous injections of another powerful vasodilator drug, sodium nitroprusside in doses sufficient to produce a similar fall in blood pressure. No alterations in the NMR spectra were observed.

4. DISCUSSION

Although many peptides can increase plasma, PRL concentrations after intravenous injection most probably act by affecting the release of dopamine from the hypothalamus. Testing the direct action of putative PRL releasing hormones on the cells of the pituitary usually requires in vitro techniques. These often produce conflicting reports: some authors have found that VIP stimulates PRL secretion in vitro [2,3,10,11] while others have not, or have only obtained some reversal of dopamine inhibition [1,12].

The data in fig.1 show that VIP enhances TRH-induced PRL secretion from tumour cells without itself causing significant hormone release. The metabolic changes accompanying these effects were visualised by NMR using a surface coil placed over the tumour which monitored changes in high energy phosphate compounds and in intracellular

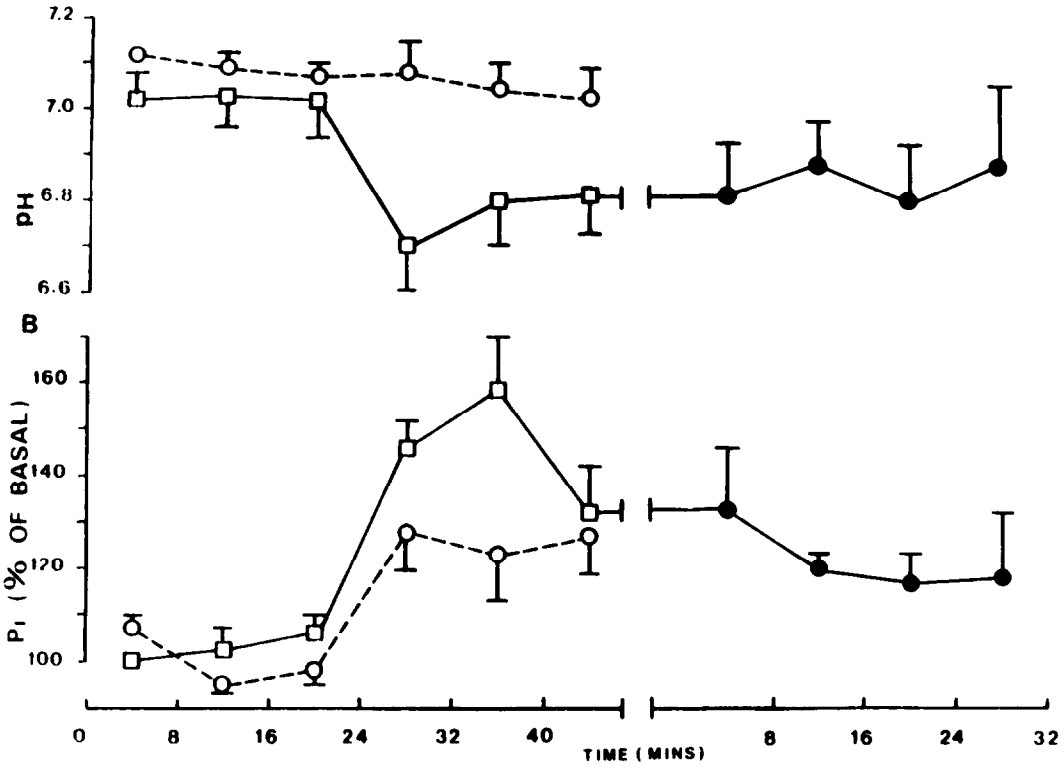

Fig.3. Quantitative changes in (A) intracellular pH and (B) inorganic phosphate of prolactin-secreting tumours following injections of VIP and/or TRH. P_i changes are given as percent change of control spectra ±SE. (□—□) VIP, (○—○) TRH, (●—●) TRH after VIP (the gap in the time scale between giving VIP and subsequently TRH varied between 4 and 20 min).

pH (fig.2). Both TRH and VIP caused depletion of PCr and a rise in P_i; VIP also caused a fall in intracellular pH. The most likely explanation for these changes is that cellular energy stores (of which PCr is the most immediately accessible) were being depleted by some energy requiring process. An alternative explanation would be that tumour blood flow was reduced by the hormone and that this ischaemia (or some other VIP-induced impairment of energy conversion) caused the depletion of cellular energy reserves. VIP, as its name implies, is vasoactive, but control experiments using the powerful vasodilator sodium nitroprusside failed to alter the NMR spectrum, suggesting that the VIP effect was not caused by a central vasodilator action. This does not rule out a local action of VIP or the release of compensatory vasoconstrictor elements induced by VIP but these explanations would not account for the similar metabolic effects induced by TRH which is not thought to have vasoactive properties. The results suggest that PRL secretion is a two-stage process. The first step (stage 1) depletes the cell's energy reserves but does not necessarily result in PRL release, whereas stage 2, release of PRL into the blood stream, has a negligible energy requirement. VIP induces stage 1 but not stage 2. TRH induces both stages but induces stage 1 less effectively than VIP at the approximately equimolar doses used. Tumours pretreated with VIP show no stage 1 response when subsequently treated with TRH, even though PRL secretion is enhanced, whereas a stage 1 response can be induced by VIP in TRH-treated tumours.

The second messengers involved in the actions of TRH and VIP have been extensively investigated. In recent years it has become clear that, although TRH-induced changes in cAMP have been measur-

ed by many authors, a change in cAMP is not essential for the acute TRH stimulation of PRL release. The second message is now thought to be a rise in intracellular Ca^{2+} (review [13]). The action of VIP in various tissues, however, seems always to be mediated by cAMP [14].

This paper provides evidence from a combination of hormonal and NMR investigations that only stage 1 of PRL release is influenced by VIP, presumably acting through cAMP. Hormone release, stage 2, is stimulated by TRH which can also, but does not invariably, induce stage 1. Since it is thought that TRH induces PRL release (i.e., stage 2) via a Ca^{2+}-mediated mechanism, its action in inducing stage 1 corresponds to the cAMP changes which are sometimes seen following the action of TRH. For example, in serum-containing medium, TRH stimulates PRL release from GH3 cells without altering cAMP, a pure stage 2 effect. In serum-free medium, on the other hand, TRH induces both PRL release and cAMP formation, i.e., both stages 1 and 2 [15]. A plausible hypothesis is that stage 1 involves an energy requiring preparation of the cell for hormone release, whereas the release of hormone in stage 2 (presumably mediated by a Ca^{2+}-induced membrane depolarisation) requires relatively little energy. VIP brings more of the hormone store of tumour cells into readiness for secretion so that subsequent TRH treatment causes a massive hormone release with little demand on cellular energy reserves.

These initial studies would appear to be the first demonstration of NMR spectral changes accompanying stimulation of an endocrine tumour, and the technique promises to be useful in explaining further intracellular events which underlie hormone secretion.

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