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VASOACTIVE INTESTINAL POLYPEPTIDE DOES NOT AFFECT THYROID FOLLICULAR CELL MEMBRANE POTENTIAL OR INPUT RESISTANCE

STEPHEN THOMAS GREEN*
Department of Physiology, The University, Dundee DD1 4HN, U.K.

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Abstract—It has been suggested that vasoactive intestinal polypeptide (VIP) has a role as a neurotransmitter and that it participates in the control of hormone release from thyroid follicles. Many hormones and neurotransmitters alter the ionic permeability properties of the plasma membrane of their target cells when they bind to their receptors. Concentrations of VIP able to elicit complex time-dependent changes in rat thyroidal cyclic nucleotide levels did not affect follicular cell membrane potential or input resistance. The suggestion is made that the findings may indicate that Ca^{2+} is not involved in the initial stages of stimulus-secretion coupling following VIP binding to its receptor on the follicular cell.

Although the adenohypophyseal hormone thyrotropin has long been recognised as the principal factor in the control of hormone release from thyroid follicles, evidence exists suggesting that the autonomic nervous system also plays a part. Adrenergic [1], cholinergic [2, 3] and non-adrenergic non-cholinergic (NCNA) nerve [4, 5] terminals impinging directly upon thyroid follicles have been identified, and a possible higher neuroanatomical and neurophysiological basis exists for the co-ordination of these neural and endocrine regulatory mechanisms since the hypothalamus is the most important structure controlling the activities both of the adenohypophysis and the autonomic nervous system [6]. Receptors specific for VIP (vasoactive intestinal polypeptide) have been identified on the follicular cell membrane [7], while VIP can induce thyroid hormone release [4, 8] and affect other aspects of the gland's metabolism [9–11]. Thus since VIP-containing nerve terminals have been demonstrated impinging directly upon the thyroid follicles of some species, including the rat and man [4], the suggestion has been advanced that intrathyroidal VIP has a role as an autonomic secretomotor neurotransmitter.

Many hormones and neurotransmitters evoke marked changes in the plasma membrane conductance of their target cells when they bind to their receptors, leading to significant alterations in cell membrane potential and input resistance [12, 13] and the presence or absence of such electrophysiological changes allows certain inferences to be drawn concerning the mechanisms of stimulus–secretion coupling operating in these cells [14, 15]. Evidence for the presence or absence of plasma membrane conductance changes occurring in the follicular cell in response to VIP has not previously been sought in detail. The present work uses the technique of continuous microelectrode recording from single cells to show that VIP, at concentrations able markedly to affect thyroidal cyclic nucleotide metabolism, does not influence follicular cell membrane potential or input resistance in the rat.

MATERIALS AND METHODS

Female Sprague–Dawley rats were killed by a blow on the head and exsanguination via the carotid arteries. The thyroid gland was removed within minutes of death. One thyroid lobe (chosen at random) was mounted in a Perspex bath and continually superfused with oxygenated Krebs–Henseleit bicarbonate-buffered physiological saline solution (for composition, see Ref. 16) at 37°, with an osmolality of 290 ± 5 mosmol/l and equilibrated at a pH of 7.4 by gassing with 5% CO_2/95% O_2. Exposure of the tissue to VIP was achieved by changing the superfusion fluid over to one in which the peptide had been dissolved to the required concentration. The method of preparation of the glass microelectrodes has been described in detail elsewhere [17, 18]: electrodes with tip resistances of between 20 and 40 MΩ were selected for use (see Ref. 14). Microelectrode tips were inserted into surface cells with the aid of a stepping motor micromanipulator (AB Transvertex) under visual control (Leitz binocular microscope, 160×). The technique and equipment used to measure and record cellular transmembrane potentials and input resistances have been described elsewhere [17]. A successful cellular impalement was defined according to the criteria outlined previously [14, 17]; a stable potential of 2 min duration was the minimum acceptable in these studies, but microelectrodes could actually be held stable within individual cells for more than an hour, allowing the nature and time-course on any electrical responses to VIP to be determined accurately.

Porcine VIP was supplied by Sigma.

* Correspondence to: Dr Stephen T. Green, University of Glasgow Department of Materia Medica, Stobhill General Hospital, Glasgow G21 3UW, U.K.

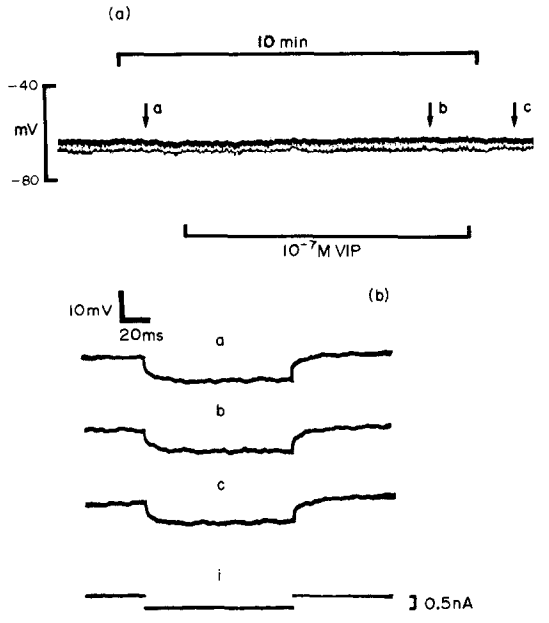

Fig. 1. Recording of membrane potential from a single thyroid follicular cell before, during and after exposure to 10^{-7} M VIP. Hyperpolarising rectangular current pulses (0.5 nA magnitude, 100 msec duration) were repetitively injected into the cell, the height of the elicited electrotonic potential changes being proportionate to the cellular input resistance at that instant in time. The electrotonic potential changes shown in (b) correspond to the points **a**, **b** and **c** indicated in (a) (see text).

RESULTS

The mean resting transmembrane potential for the follicular cell in this study of -67.4 ± 7.5 (SD) mV (N = 11) agrees well with the findings in earlier work from this and other laboratories [17–20]. Figure 1a shows a membrane potential recording from a single cell into which hyperpolarising current pulses of 100 msec duration are being injected. Initially, the tissue is exposed to a continuous flow of control solution, following which (as indicated by the bar) the solution is switched over to one containing VIP 10^{-7} M. Over a period of 7.5 min, there is no change in membrane potential, and there is no change when the flow of control solution is restored. In addition, comparison of oscillographs a, b and c (taken at the points marked by arrows on Fig. 1a) reveals that there is no effect on input resistance (see Fig. 1b). Seven similar traces using 10^{-7} M VIP were obtained, while 10^{-8} M (N = 2) and 10^{-6} M (N = 1) VIP likewise failed to affect membrane conductance: the use of hyperpolarising or depolarising current pulses did not affect this finding, while earlier work has shown that the resting current–voltage relationship for the rat thyroid follicular cell is linear over a wide range of membrane potentials (from −20 mV to −90 mV) indicating that this cell membrane does not possess voltage-activated ionic channels [17]. In this study, all results were obtained using the thyroids of different rats.

In order to establish that the VIP employed in this study was able to exert metabolic effects upon the rat thyroid, parallel studies investigating the effects of 10^{-7} M VIP on thyroidal cyclic AMP and cyclic GMP levels were performed in this laboratory; these results have been presented elsewhere (see Ref. 9). Figure 2 is constructed from this data and it is clear from this both that 10^{-7} M VIP does exert metabolic effects upon this preparation within the time span examined electrophysiologically and that the biochemical response of the tissue to VIP is complex and time-dependent.

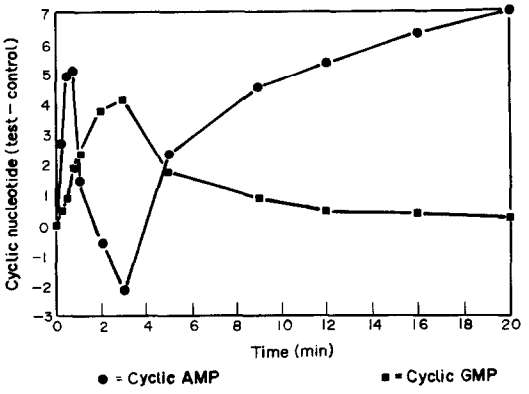

Fig. 2. Changes in thyroidal levels of cyclic AMP and cyclic GMP in response to 10^{-7} M VIP (see text). Cyclic nucleotide levels are measured in pmol·(mg protein)$^{-1}$

DISCUSSION

This is the first report describing the electrophysiological consequences of VIP interacting with its membrane-bound receptor in thyroid cells, and demonstrates that VIP does not affect follicular cell membrane potential or input resistance, indicating in turn that neither plasma membrane or gap junctional conductance is altered in response to VIP. In view of the fact that more than 95% of the total cellular mass of the thyroid is made up of follicular cells [21] while the potentials recorded from the follicular lumens are considerably lower than those recorded from the cells [20], it follows that there is a high degree of confidence that the cells being recorded from in this study are indeed follicular cells. It is clear from the cyclic nucleotide data that VIP is able to elicit biochemical responses within the cells over the time period observed electrophysiologically in Fig. 1, while it has been shown in the dog that thyrotropin (which has a similar temporal effect on tissue cyclic nucleotide levels to that elicited by VIP [9, 19]) causes apical pseudoped formation within 2 min and thyroid hormone release takes place within 10 min [22]. Electrical field stimulation (FS), a technique which induces intrinsic nerve terminals within a tissue to release their neurotransmitters, causes thyroxine release from the rat thyroid within 7 min [19]. It has been shown, using the continuous single cell recording technique, that thyrotropin [18] and FS [19] have no effect on rat thyroid follicular cell membrane conductance while exerting biochemical effects upon the gland, and the FS results suggest that any neurotransmitters released from intrathyroidal

nerve terminals (this possibly includes VIP [4, 9]) do not affect follicular cell membrane conductance [19].

To understand the significance of these observations in the follicular cell, it is important to consider some general aspects of stimulus–secretion coupling. Many gland cells use exocytosis as their vehicle for secretion, exocytosis being a Ca^{2+}-dependent process [23], while it has been suggested that a change in plasma membrane ionic permeability in response to secretagogue–receptor binding is in some way an important requirement for exocytosis to be able to occur [14, 15]. Conversely, in some tissues such as the exocrine pancreas, salivary glands and lacrimal gland, it is clear that the Ca^{2+} ion is involved in some way as a second messenger in the generation of the observed plasma membrane conductance changes in response to receptor activation [24]. Since thyroid hormones are not transferred to the exterior from the cellular interior by means of an exocytotic process [25], it follows that no requirement exists to postulate that thyrotropin or any other stimulatory molecule (such as VIP) should cause changes in follicular cell membrane potential or input resistance. Both secretin [26] and VIP [27] are able to induce enzyme secretion from pancreatic acinar cells (almost certainly via a non-Ca^{2+}-dependent process [15, 27]) without affecting acinar cell membrane conductance: similarly, dibutyryl cyclic AMP evokes vigorous non-Ca^{2+}-dependent exocytosis of amylase from the mouse parotid, yet this is unaccompanied by any changes in acinar cell input resistance [28]. This suggests, in turn, the possibility that thyroid hormone release in response to VIP or thyrotropin may likewise occur by means of a Ca^{2+}-independent process: this notion does not conflict with the idea that Ca^{2+} may be important in the control of other thyroidal processes, such as stimulant-evoked glucose C-1 oxidation and protein iodination [29].

As adrenergic [30], cholinergic [3] and VIP-containing [4, 8] nerves have been found in the human thyroid, the present observations could seem important as therapeutic implications may be involved.

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