volts. Shortly thereafter and close to the dusk meridian, the ion flow patterns took on a highly erratic character and alternated with high isotropic fluxes.

Five additional examples of directed ion flow in the magnetosphere during magnetic disturbances were included in the data from the ATS-1 ion detector. Of these, three resemble the event of 13 to 14 January 1967; they show flow in the quadrant from noon to dusk with the flow being predominantly toward the sun; however, they do not possess the remarkable boundary-crossing feature.

Another of the examples takes place during a magnetic sudden commencement. This shows flow near the noon meridian initially toward the earth, after which a more complex time-varying pattern occurs.

Statistically significant anisotropies have also been found in data averaged over 3 hours from a few of the approximately 30 magnetically quiet days examined. The flow pattern seen on these days is not, in general, repeatable from day to day. However, trends in the data appear to support the magnetic storm pattern associated with nonsudden commencement events—flow toward the sun in the quadrant from noon to dusk.

Thus large-scale motion of the magnetospheric thermal plasma exists at least during certain intervals of both high and low magnetic activity. The flow pattern observed is consistent with present models of magnetospheric convection for the day hours of local time. The pattern for the nightside hours has not been established. The work of Carpenter (*9*), when considered together with the popular convection models, probably points to the continuous presence of large-scale electric fields with the ion gusts accounted for by a varying ion number density.

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## Thyroid-Stimulating Hormone and Prostaglandin $E_1$ Stimulation of Cyclic 3′,5′-Adenosine Monophosphate in Thyroid Slices

Abstract. *Thyroid-stimulating hormone increased the cyclic 3′,5′-adenosine monophosphate concentration in dog thyroid slices during a 1-minute incubation period and produced a maximum effect soon thereafter. The elevation persisted for at least 30 minutes. The concentrations of the cyclic 3′,5′-adenosine monophosphate increased as the TSH concentration was increased from 0.125 to 50 milliunits per milliliter. Prostaglandin $E_1$, which increases glucose oxidation in dog thyroid slices, also increased the concentration of cyclic 3′,5′-adenosine monophosphate. Although sodium fluoride stimulates thyroid adenyl cyclase, it did not increase concentration of cyclic 3′,5′-adenosine monophosphate. Carbamylcholine and menadiol sodium diphosphate augment glucose oxidation in dog thyroid slices but do not change concentrations of cyclic 3′,5′-adenosine monophosphate.*

Current evidence suggests that thyroid-stimulating hormone (TSH) regulates thyroid gland metabolism as a consequence of stimulation of the enzyme adenyl cyclase and generation of cyclic 3′,5′-adenosine monophosphate (AMP). Thyroid-stimulating hormone rapidly increased adenyl cyclase activity in beef thyroid homogenate (*1*), and the metabolic and morphologic effects of TSH on the thyroid have been reproduced with dibutyryl cyclic 3′,5′-AMP (*2*, *3*). Gilman and Rall reported that TSH increased cyclic 3′,5′-AMP concentrations in beef thyroid slices (*4*). Many other substances besides TSH also stimulate glucose oxidation in thyroid slices, and a mechanism involving cyclic 3′,5′-AMP has been implicated for some. Sodium fluoride augments glucose oxidation (*5*, *6*) and stimulates adenyl cyclase in thyroid homogenate (*6*). We have demonstrated that prostaglandin $E_1$ increased glucose oxidation in thyroid slices but, differing from TSH, did not stimulate incorporation of $^{32}P$ into phospholipid (*7*). Although acetylcholine and menadione both augmented glucose oxidation in thyroid (*8*), acetylcholine did not reproduce TSH stimulation of adenyl cyclase in thyroid homogenate (*1*). Although the hypothesis that cyclic 3′,5′-AMP is the intracellular mediator of hormone action is most attractive (*9*), the available data indicate that similar effects on thyroid metabolism may be produced by other mechanisms.

In order to obtain more direct evidence for the role of cyclic 3′,5′-AMP in the control of thyroid gland function, we have studied the effects of various substances on the concentration of this nucleotide. Dog thyroid slices weighing between 20 and 60 mg were prepared and incubated in Krebs-Ringer bicarbonate buffer (*10*). After an initial incubation for 20 minutes in 2 ml of buffer containing 2 mg of glucose, the slices were transferred to flasks containing the same buffer and the appropriate hormone or substance. The time of this second incubation is indicated in the tables. Cyclic 3′,5′-AMP was measured by a modification (*11*) of the method of Breckenridge (*12*). After the second incubation, tissue slices were immediately frozen between blocks of dry ice. The frozen slice was homogenized in 5 percent trichloroacetic acid; the trichloroacetic acid was removed by ether extraction. Cyclic 3′,5′-AMP was then separated from adenosine triphosphate (ATP), adenosine diphosphate (ADP), and 5-AMP by barium hydroxide and zinc sulfate precipitation and column chromatography with Dowex-50 (*13*). The cyclic nucleotide was converted to ATP by incubation with phosphodiesterase, myokinase, and pyruvate kinase. The resulting ATP was assayed with glucose-1-$^{14}C$ (*14*). This method was modified to include pyruvate kinase and phosphoenolpyruvate so that the ADP formed would be recycled to generate more ATP. The sensitivity of this method is $10^{-12}$ mole. Cyclic 3′,5′-AMP labeled with tritium was used as an in-

Table 1. Time course of TSH stimulation in vitro of cyclic 3′,5′-AMP in dog thyroid slices. Four experiments, each with a different dog thyroid, are shown. The TSH concentration was 10 milliunit/ml. The results are the averages of duplicate determinations on each slice.

| Treatment | Cyclic 3′,5′-AMP (pmole/g) after incubation with TSH | | | |
|---|---|---|---|---|
| | 1 min | 3 min | 10 min | 30 min |
| None | 1512 | 1247 | 2026 | 682 |
| TSH | 2983 | 7194 | 8679 | 1700 |
| None | 580 | | 576 | |
| TSH | 3707 | | 10990 | |
| None | | | 557 | 386 |
| TSH | | | 4254 | 3840 |
| None | | 1478 | | 1096 |
| TSH | | 5790 | | 6920 |

Table 2. The effect of TSH concentration on cyclic 3′,5′-AMP in dog thyroid slices. Two separate experiments, each with a different dog thyroid, are shown. The time of incubation with TSH was 10 minutes. In some instances the results are the averages of duplicate determinations in two slices, while in others they are the average of duplicate determinations on a single slice.

| Exp. No. | Cyclic 3′,5′-AMP (pmole/g) after treatment with TSH (milliunit/ml) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 0.125 | 0.5 | 1.5 | 5 | 12.5 | 50 |
| 1 | 471 | 739 | | 1056 | 3387 | 4125 | 4815 |
| 2 | 576 | 648 | 851 | 2822 | 19000 | | |

ternal standard to monitor recovery throughout the procedure.

The data in Table 1 indicate that 10 milliunits of TSH per milliliter increased the cyclic 3′,5′-AMP concentration in dog thyroid slices after 1 minute of incubation. The rapidity of the increase in cyclic 3′,5′-AMP is consistent with the observation that TSH increased adenyl cyclase activity in beef thyroid homogenate within 30 seconds of its addition (*1*). Gilman and Rall reported that maximum effects of TSH, in the presence of theophylline, were observed during a 6-minute incubation (*4*). The minimum dose of TSH which increased the cyclic 3′,5′-AMP concentrations in thyroid slices was between 125 and 500 microunit/ml (Table 2). Marked effects were observed with doses of TSH between 1.5 and 5 milliunit/ml and further elevation was produced by higher doses up to 50 milliunit/ml. Although 50 microunits of TSH per milliliter has been reported to increase glucose oxidation in dog thyroid slices (*15*) and adenyl cyclase in beef thyroid homogenate (*1*), this dose did not increase the cyclic nucleotide concentration. The amounts of TSH which augment cyclic 3′,5′-AMP are certainly similar to those which have been reported to stimulate other contributors to thyroid gland metabolism.

Prostaglandin $E_1$ mimics the effect of TSH on glucose oxidation in dog thyroid slices (*7*). The data in Table 3 indicate that this substance also augments cyclic 3′,5′-AMP concentrations. Although in this respect its effect was similar to that of TSH, prostaglandin did not reproduce the action of TSH on incorporation of $^{32}P$ into phospholipid (*7*). This dissociation of effects raises the possibility that stimulation of $^{32}P$ into phospholipids may be mediated by a mechanism not involving cyclic 3′,5′-AMP. The observation that dibutyryl cyclic 3′,5′-AMP did not increase incorporation of $^{32}P$ into dog thyroid slices would be consistent with this (*2*). Prostaglandin has also been reported to elevate cyclic nucleotide concentration in several other tissues (*16*). Acetylcholine stimulates glucose oxidation, $^{32}P$ incorporation into phospholipids (*17*), and phosphorylase activity (*18*) in dog thyroid slices; but carbamylcholine did not increase cyclic 3′,5′-AMP. This is consistent with the observation of Pastan and Macchia that acetylcholine did not activate adenyl cyclase activity in beef thyroid homogenate (*1*). It would thus appear that acetylcholine stimulation of glucose oxidation, phospholipid synthesis, and phosphorylase may reflect a mechanism not involving cyclic 3′,5′-AMP. Menadione also increases glucose oxidation in dog thyroid slices, but menadiol sodium diphosphate had no effect on cyclic 3′,5′-AMP. The stimulation of glucose oxidation produced by menadione has been attributed to its ability to oxidize reduced nicotinamide adenine dinucleotide phosphate (NADPH) (*8*). Such a mechanism would not have to involve cyclic 3′,5′-AMP.

Sodium fluoride is a potent stimulator of adenyl cyclase in thyroid and several other tissues (*6*, *19*). Furthermore it increases glucose oxidation in thyroid slices (*5*, *6*). It therefore was somewhat surprising to find that it did not increase cyclic 3′5′-AMP in dog thyroid slices (Table 3). A somewhat analogous situation has been described in an adrenal tumor (*20*). Although adrenocorticotropic hormone increased adenyl cyclase in tumor homogenates, it did not elevate cyclic 3′5′-AMP in tumor slices. The explanation for this discrepancy is not apparent. However, such results emphasize the point that the expression of hormonal effects in tissues may be much more complicated than merely stimulating adenyl cyclase activity and generating cyclic 3′,5′-AMP.

Table 3. Effect of TSH and other substances on cyclic 3′,5′-AMP in dog thyroid slices. Two separate experiments, each with a different dog thyroid, are shown. The time of incubation with each substance was 3 minutes. The results are averages of duplicate determinations on each slice.

| Treatment | | Cyclic 3′,5′-AMP (pmole/g) | |
|---|---|---|---|
| Substance | Concentration | Exp. 1 | Exp. 2 |
| None | | 601 | 861 |
| TSH | 10 milliunit/ml | 3626 | 2090 |
| Prostaglandin $E_1$ | 30 $\mu$g/ml | 5571 | 1817 |
| Carbamylcholine | $1 \times 10^{-5} M$ | 808 | 954 |
| Menadiol | $3 \times 10^{-5} M$ | 433 | 637 |
| NaF | $1 \times 10^{-2} M$ | 304 | 872 |

Although the mechanism by which cyclic 3′,5′-AMP causes alterations in thyroid gland metabolism and morphology still remain to be clarified, our results are consistent with a very early, perhaps even primary, action of TSH, to increase cyclic 3′,5′-AMP in the thyroid. Effects of fluoride and other substances which influence thyroid gland metabolism are probably mediated by a different mechanism from that of TSH and may have no relation to concentrations of cyclic 3′,5′-AMP in the thyroid.

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