with a linear response up to 0.2 milliunits. In the experiments reported here, various dilutions of the experimental fluids were used depending on the amounts of expected TSH released in the fluids, this having been appreciated usually in preliminary experiments. As shown in the Table, the results are expressed as ratios of the amounts of TSH released by the experimental pituitaries (T, treatment) over that released by the control pituitaries (N, normal). These ratios are obtained by complete 4-point assays between T and N fluids; thus true confidence limits can be calculated for these ratios. As an indication of the amounts of TSH involved in these experiments, *approximate* values (milliunits of TSH) released during the incubation are also reported from calculations in 3-point assays.

Duration of incubations and times of addition of treatments are reported in details in the Table. The 4 multiple experiments reported here with somewhat different designs gave confirmatory results. From the first experiment (protocol No. 8009) it can be seen that incubation in $Ca^{++}$-free medium did not modify, per se, secretion of TSH; addition of TRF stimulated acute release of TSH from the tissues incubated in Krebs-Ringer, whereas the effect was completely prevented in the pituitaries incubated in $Ca^{++}$-free medium. The results obtained from the second experiment (protocol No. 8017) with a similar design, were identical. In the third and fourth experiments (protocol No. 8037 and No. 8040), with closely related designs, the response to TRF usually observed was obtained in the control group, whereas it was again greatly diminished in absence of $Ca^{++}$. The pituitaries were then re-incubated in regular Krebs-Ringer; when TRF was added to the tissue previously incubated in $Ca^{++}$-free medium, TSH-release was stimulated, thus demonstrating that the prior altered response to TRF was not due to irreversible alterations to the pituitary tissue caused by the prolonged incubation in absence of $Ca^{++}$.

*Discussion.* It would appear from the results presented here that $Ca^{++}$ plays an important and necessary role in the intimate mechanisms whereby hypothalamic TRF stimulates acutely the release of TSH. Similar results in the past have been interpreted as involving $Ca^{++}$ in the 'stimulus-secretion coupling'[9,10] for electrical stimulation, high K environment, locally elevated concentrations of acetylcholine and the secretory activity of the tissues to which these various electrical or chemical informations were applied[9-11]. The results reported here are compatible with the concept that the same hypothesis may apply to the adenohypophysial tissue and a specific mediator of its secretion, one of the hypothalamic releasing factors. A possible role of $Ca^{++}$ as implicated in the secretion of the adenohypophysis had been anticipated some years ago by DOUGLAS and POISNER[12,13].

*Résumé.* L'incubation in vitro de fragments d'hypophyse (rat) dans le liquide de Krebs-Ringer sans calcium ($Ca^{++}$) inhibe l'action du facteur hypothalamique TRF (TSH-releasing factor) qui normalement stimule la sécrétion de l'hormone thyréotrope (TSH). La ré-introduction de l'ion $Ca^{++}$ (2.54 m*M*) rétablit la réponse (sécrétion de TSH) au facteur TRF.

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## Potassium-Induced Stimulation of Thyrotropin Release in vitro. Requirement for Presence of Calcium and Inhibition by Thyroxine

We have shown in a previous note that calcium ions ($Ca^{++}$) are necessary for the hypothalamic hormone TRF (thyrotropin-releasing factor) to stimulate in vitro the secretion of thyrotropin (TSH)[1]. With availability of the above results and in keeping with the hypothesis presented in [1] that led us to investigate the possible requirement for $Ca^{++}$ on the action of TRF, we decided to study the effects of elevating potassium ($K^+$) content in the fluid of the in vitro pituitary incubation, on TSH secretion. The results presented below will demonstrate that $K^+$ can stimulate the in vitro secretion of TSH, that this effect, like that of TRF, requires the presence of $Ca^{++}$ and also that it can be inhibited by pre-incubation with thyroxine.

*Materials and methods.* The methods for incubation of the pituitaries, for the bioassays of TSH and their statistical analysis, for the characterization of the TRF used here, have all been described in detail in a previous note[1]. The solutions of L-thyroxine Na (Calbiochem. Los Angeles, California) used here were prepared freshly in alkalinized 0.9% NaCl for each experiment, at a concentration of 27.0 $\mu$g/ml. At the end of the incubation period, fluids were diluted with the proper medium so that the same concentrations of electrolytes were achieved in the fluids from controls and treated tissues to be injected in the animals used in the TSH assay.

*Results.* (1) *Effects of high $K^+$ on TSH-release.* Several experiments were conducted with various designs as reported in the Table. All studies show that when the $K^+$ molarity of the incubation fluid is raised to 25 meq/l or above (protocol Nos. 7973, 7990a, 7998, 8029, 8002a), release of TSH takes place.

(2) *Effects of high $K^+$ on TSH-release in absence of $Ca^{++}$.* The release of TSH induced by elevated $K^+$ (25 meq/l) is completely abolished when $Ca^{++}$ is omitted from the incubation fluid (protocol Nos. 8017, 8026). The response

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| Time in min | Treatments | | TSH released | TSH released mU/incubation | |
|---|---|---|---|---|---|
| | N | T | T/N | N | T |
| Protocol No. 7973 | | | | | |
| 0 | KR | KR | | | |
| 20 | KR | KR | | | |
| 40 | KR | 100 meq $K^+/_l$ | 5.63 (2.79–24.5) | < 0.6 | 3.3 |
| 55 end | | | | | |
| Protocol No. 7990a | | | | | |
| 0 | KR | KR | | | |
| 10 | KR | KR | | | |
| 40 | KR | KR | | | |
| 60 | KR | 50 meq $K^+/_l$ | 3.74 (2.29–9.31) | < 0.6 | 3.10 |
| 75 end | | | | | |
| Protocol No. 7998 | | | | | |
| 0 | KR | KR | | | |
| 15 | KR | KR | | | |
| 60 | KR | 25 meq $K^+/_l$ | 6.20 (2.76–241.8) | < 0.6 | 3.2 |
| 75 end | | | | | |
| Protocol No. 8002a | | | | | |
| 0 | KR | KR | | | |
| 20 | KR | KR | | | |
| 60 | KR | 25 meq $K^+/_l$ | 2.76 (1.74–6.43) | < 0.6 | 1.12 |
| 75 end | | | | | |
| Protocol No. 8017 | | | | | |
| 0 | KR | O $Ca^{++}$ | | | |
| 10 | KR | O $Ca^{++}$ | | | |
| 30 | KR | O $Ca^{++}$ | | | |
| 50 | KR | O $Ca^{++}$ | | | |
| 75 | 25 meq $K^+/_l$ | O $Ca^{++}$ + 25 meq $K^+/_l$ | 0.15 (0.016–0.33) | 2.70 | < 0.6 |
| 90 end | | | | | |
| Protocol No. 8026 | | | | | |
| 0 | KR | O $Ca^{++}$ | | | |
| 10 | KR | O $Ca^{++}$ | | | |
| 30 | KR | O $Ca^{++}$ | | | |
| 60 | 25 meq $K^+/_l$ | O $Ca^{++}$ + 25 meq $K^+/_l$ | 0.53 (0.22–0.91) | 0.8 | < 0.03 |
| 80 | KR | KR | | | |
| 110 | 25 meq $K^+/_l$ | 25 meq $K^+/_l$ | | 1.4 | 1.6 |
| | | | | 1.47 (0.9–2.7) [b] | 3.60 (2.1–9.3) [a] |
| 130 end | | | | | |
| Protocol No. 7990b | | | | | |
| 0 | KR | KR + $T_4$ | | | |
| 10 | KR | KR + $T_4$ | | | |
| 40 | KR | KR + $T_4$ | | | |
| 60 | 50 meq $K^+/_l$ | 50 meq $K^+/_l$ + $T_4$ | 0.64 (0.43–0.82) | 4.20 | 1.44 |
| 75 end | | | | | |
| Protocol No. 8002b | | | | | |
| 0 | KR | KR + $T_4$ | | | |
| 20 | KR | KR + $T_4$ | | | |
| 60 | 25 meq $K^+/_l$ | 25 meq $K^+/_l$ + $T_4$ | 0.44 (0.20–0.62) | 2.0 | < 0.6 |
| 75 end | | | | | |
| Protocol No. 8048b | | | | | |
| 0 | KR | KR + $T_4$ | | | |
| 15 | KR | KR + $T_4$ | | | |
| 60 | 25 meq $K^+/_l$ | 25 meq $K^+/_l$ + $T_4$ | 0.31 (0.10–0.53) | 3.30 | 0.56 |
| 80 end | | | | | |

N, T, normal control, treated experimental pituitary, respectively. KR, regular Krebs-Ringer bicarbonate glucose medium. O $Ca^{++}$, incubation medium free of $Ca^{++}$. $T_4$, L-thyroxine Na. Treatments, indicates the various additions or fluids added at the time indicated. TSH released, corresponds to the amount of TSH measured in the incubation fluids *at the end* of the incubation period starting at the time shown in the same horizontal row. [a], ratio with its true confidence limits of the amounts of TSH released by the same pituitaries (T) in response to $K^+$ in presence of $Ca^{++}$ to that observed in $Ca^{++}$-free medium. [b], ratio with its true confidence limits of the amounts of TSH released by the same pituitaries (N) in response to $K^+$ in the successive exposures.

(release of TSH) to elevated $K^+$ is re-established however when $Ca^{++}$ is reintroduced in the incubation fluid (protocol No. 8026), thus showing that the absence of response in absence of $Ca^{++}$ is not due to some irreversible cellular damage.

(3) *Effects of* L-*thyroxine Na (T4) on TSH-release induced by elevated* $K^+$. In the first experiment (protocol No. 7990b), a large dose of T4 (10 µg/ml) prevented the release of TSH induced by 50 meq $K^+$/l. In 2 subsequent experiments (protocol Nos. 8002b, 8048), pre-incubation of the pituitary tissues with 1.25 µg T4/ml prevented the release of TSH induced by 25 meq $K^+$/l.

*Discussion.* Perhaps the most striking aspect of the observations reported in this note is to be found in the similarities between the effects (TSH release) of $K^+$ and TRF: both require the presence of $Ca^{++}$ for their activity in releasing TSH and thyroxine can inhibit the TSH release induced by either $K^+$ or TRF.

The results reported here are consistent with one of our current hypotheses that a decrease in the membrane potential of the thyrotroph cells of the adenohypophysis may be involved in the action of TRF to stimulate release of TSH.

*Addendum.* Since these data were submitted for publication, evidence has been obtained that elevated $K^+$ stimulated in vitro secretion of ACTH and LH, as measured by bioassays; also, SAMLI and GESCHWIND have reported (Prog. 49th Meet. Endocr. Soc., p. 58) that elevated $K^+$ stimulated secretion of LH, as measured by immunoassay[2].

*Résumé.* L'augmentation de la concentration en potassium ($K^+$) à 25 meq/l et au delà, du liquide dans lequel des hypophyses de rat sont incubées in vitro stimule la sécrétion de l'hormone thyréotrope (TSH). La présence de l'ion calcium ($Ca^{++}$) est nécessaire à cet effet de $K^+$; l'incubation dans un milieu sans $Ca^{++}$ empêche l'effet (sécrétion de TSH) de $K^+$, qui est rétabli quand on réintroduit $Ca^{++}$ dans le liquide d'incubation. La stimulation de la sécrétion de TSH due à $K^+$ est inhibée par la thyroxine. Les effets de $K^+$ sur la sécrétion de TSH sont identiques à ceux de TRF (TSH-releasing factor) hypothalamique, en ce qui concerne la présence de $Ca^{++}$ et l'inhibition par la thyroxine.

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## A Qualitative Difference between Certain H-2 and Non-H-2 Antigens Responsible for a Similar Graft-Rejection Rate

The establishment of congenic (isogenic resistant) lines of mice[1] made it possible to compare the relative strength of antigens controlled by individual histocompatible (H) loci. By expressing the antigenic strength in terms of graft rejection rate, one strong and several weak H-loci were defined. The strong locus designated H-2 is a complex pseudoallelic series whose individual pseudoalleles control several antigenic specificities[2]. In the usual H-2 incompatible donor-host combinations the time taken for the rejection of first-set skin grafts does not exceed 14 days. When, however, the spectrum of H-2 antigens representing the target of allograft reaction is limited, the graft rejection time may be prolonged[3]. In contrast to this, a cumulative effect of weak (non-H2) loci was described[4].

MCKHANN[5] compared strong and weak histocompatibility barriers by means of graft sensitivity induced by spleen cells. In the H-2 system, sensitization appeared rapidly and persisted for a shorter time than in H-3 where it appeared more slowly. The ease of tolerance-induction usually appears inversely proportional to the graft rejection rate. In adult mice, induction of tolerance across H-2 barrier requires a prolonged administration of large doses of spleen cells[6], whereas a single dose is sufficient when H-3 barrier is to be overridden[7].

We were interested in whether the H-2 and non-H-2 antigens are qualitatively similar when chosen so that they represent a comparable histocompatibility barrier.

In a combination differing at 2 'weak' loci, i.e. donor B10.LP ($H\text{-}1^c$, $H\text{-}2^b$, $H\text{-}3^b$) and recipient B10.BY ($H\text{-}1^d$, $H\text{-}2^b$, $H\text{-}3^a$) the average skin graft rejection time is about 17 days, whereas in a particular H-2 incompatible combination (i.e. donor B10.A[RII]) ($H\text{-}2^h$) and recipient (B10.D2 × B10.BR)F1 ($H\text{-}2^d/H\text{-}2^k$) where according to the data given by SNELL et al.[2], the antigenic target is presumably only antigen 2 ($D^b$), it amounts to 20 days.

The skin grafting technique was in principle that described by BILLINGHAM and MEDAWAR[8]; the first macroscopic examination followed on day 7 postgrafting and then daily. The day of appearence of an essential cosmetic defect (which does not tend to improve on following days) is given as the day of graft rejection. The criterion of a 'permanent tolerance' is the survival of second skin graft on recipient tolerating the first graft for more than 100 days.

Recipients of skin grafts were pretreated with a single dose of $5 \times 10^7$ or $8 \times 10^8$ of viable spleen cells. Test grafting followed on day 4, 10 or 40 after the lower dose and on day 3 after the higher one. In the case of H-2 incompatibility, the dose of $5 \times 10^7$ cells led to an accelerated rejection of 2 out of 5 grafts made on the fourth day and of all grafts made 10 and 40 days after the injection. In the (H-1 + H-3) system, a similar dose of spleen cells

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