Posterior pituitary vasopressin content in rats homozygous and heterozygous for hereditary diabetes insipidus as measured by radio-immunoassay and bioassay

| | Body weight (g) | Posterior pituitary weight (mg) | Vasopressin (mU/posterior pituitary) | | Correlation RIA/bioassay ($r$) |
|---|---|---|---|---|---|
| | | | RIA | Bioassay | |
| Homozygous | 243 ± 6 | 1.24 ± 0.07 | 8.2 ± 2.0 | 11.5 ± 2.8 | 0.83 ($p < 0.05$) (7) |
| Heterozygous | 283 ± 7 | 0.84 ± 0.03 | 163.0 ± 22.7 | 169.0 ± 27.4 | 0.97 ($p < 0.01$) (7) |

Values are mean ± S.E.M. Numbers of animals in parentheses.

rats, the vasopressin content was very low as measured by radio-immunoassay. In these samples, a $2 \times 2$ pressor assay was not feasible and a $2 \times 1$ assay was performed. Immunoassay of aliquots was performed in triple on 1:25 dilutions of pituitary homogenates of homozygous rats and on 1:250 dilutions of those of heterozygous animals.

Although body weight of the homozygous rats was less than that of the heterozygous ones, the posterior pituitary weight was significantly higher in the former group (Table) confirming the data of ARIMURA, SAWANO, REDDING & SCHALLY[5].

Vasopressin content of pituitaries of homozygous rats was extremely low compared to that of heterozygous animals as measured by bioassay as well as immunoassay (Table). A close correlation was found when pituitary vasopressin content by immunoassay ($Y$) was plotted against this content by bioassay ($X$). For heterozygous animals $r = 0.97$ ($p < 0.01$) ($Y = 1.2\,X - 29.9$) and for homozygous animals $r = 0.83$ ($p < 0.05$) ($Y = 1.2\,X + 2.0$). These results are in good agreement with those of MOSES and MILLER[1] and of VALTIN et al.[6].

Oxytocin content of the homozygous D.I. rats is greatly reduced as compared to that of heterozygous animals[6]. Moreover this oxytocin content contributes only to a small degree (< 2%) to the vasopressin-like activity in the posterior pituitary of homozygous D.I. rats as determined by blood pressure bioassay[7]. Additionally the dilution curves obtained with aliquots of pituitary homogenates in the radio-immunoassay were parallel to the AVP standard curves, in contrast with those obtained with oxytocin. Thus it is unlikely that oxytocin contributes significantly to the vasopressin-like activity that is found in posterior pituitaries of Brattleboro rats.

In the homozygous D.I. rats of the Brattleboro strain, posterior pituitary vasopressin-like activity is minimal as compared to that of heterozygous animals. The radio-immunoassay of AVP described, represents a sensitive method for the quantitative measurement of biologically active vasopressin[8,9].

*Zusammenfassung.* Die Empfindlichkeit und Zuverlässigkeit eines neu entwickelten Radioimmunotests für Vasopressin wurde durch Messungen des Vasopressinspiegels im Hypophysenhinterlappen von homozygoten und heterozygoten Ratten für angeborenen Diabetes insipidus geprüft und eine gute Korrelation zwischen dem biologisch und radioimmunologisch gemessenen Vasopressingehalt der Hypophyse nachgewiesen.

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## The Effect of Theophylline on Oxytocin Induced Contractions in the Chronically Catheterized Pregnant Rabbit

The sensitivity of the pregnant rabbit myometrium to intra-aortic oxytocin infusion increases as the circulating plasma progesterone concentration falls[1,2]. SMITH, ABEL and NATHANIELSZ[2] have described an experimental preparation in which catheters are introduced into the femoral artery and vein of a 21 day pregnant rabbit under nembutal anaesthesia. The catheters are advanced cranially until their tips lie in the aorta and vena cava, above the level of the ovarian blood vessels. If the animals are infused continuously with saline they deliver live litters approximately 185 h after operation, whereas the infusion of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) at doses of

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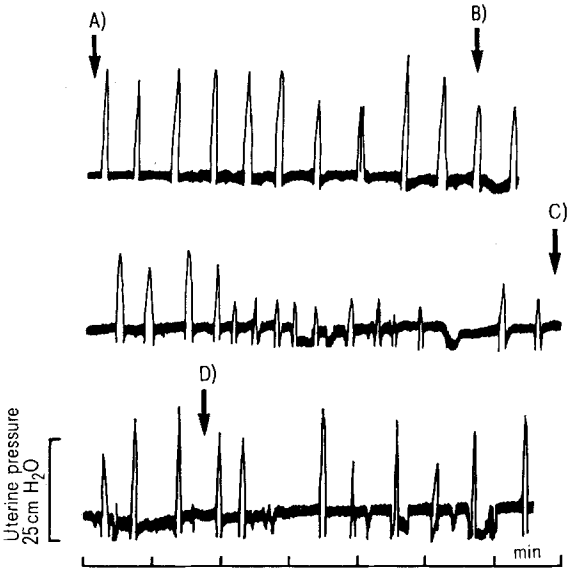


Fig. 1. Demonstration of the effect of Theophylline on oxytocin induced uterine contractions. The infusion rate throughout the experiment was 1 ml/min. A) Infusion of 10 m*U* oxytocin/ml. B) Infusion of 10 m*U* oxytocin/ml in 20 m*M* Theophylline. C) Infusion of 10 m*U* oxytocin alone. At time D) the delivery of 1 foetus occurred. The trace is continuous.

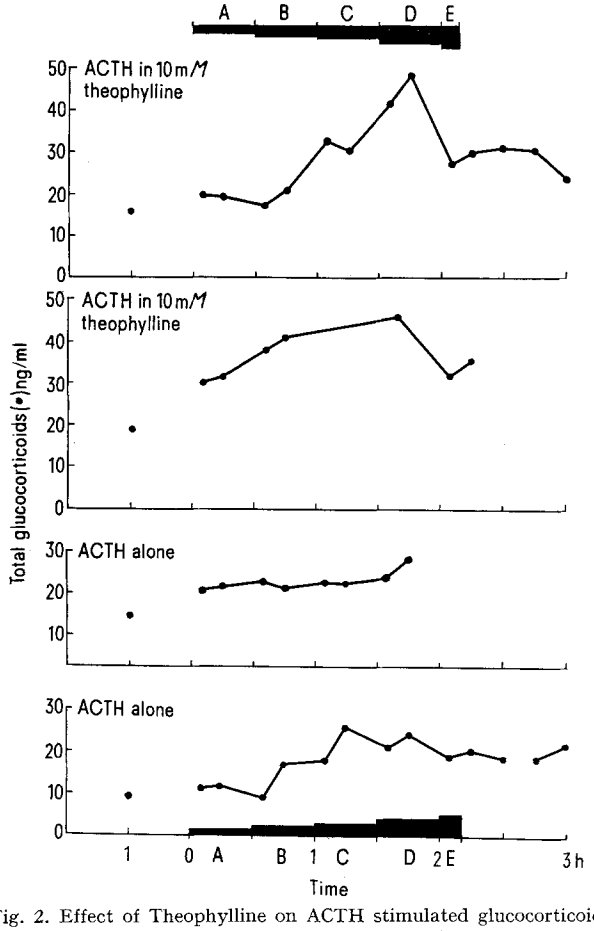


Fig. 2. Effect of Theophylline on ACTH stimulated glucocorticoid secretion. Total plasma glucocorticoid concentration was estimated on unextracted plasma using a 2.5% horse plasma stock solution[7]. The histograms represent the duration of ACTH infusion at the following doses: A) 0.2 ng/kg/min. B) 2.0 ng/kg/min. C) 0.02 μg/kg/min. D) 0.20 μg/kg/min. E) 2.0 μg/kg/min. In 2 of the 4 animals ACTH was infused in the presence of 10 m*M* Theophylline.

0.01–2.25 μg/h results in premature delivery in less than 80 h. At high infusion rates, the $PGF_{2\alpha}$ only need be administered for 8 h to produce parturition 44 h later[3].

When $PGF_{2\alpha}$ is infused for 11–16 h and then discontinued there is a gradual increase in the sensitivity of the myometrium to oxytocin and $PGF_{2\alpha}$ as monitored by an intrauterine pressure transducer operating on radiotelemetric principles[1,2]. The effect of short duration infusions of theophylline on the myometrial responsiveness to oxytocin have been investigated in this experimental model.

Rabbits were catheterized and a pressure transducer placed in one uterine horn on day 21 of pregnancy. They were then infused intraaortically with 1 μg $PGF_{2\alpha}$/h for 16 h. All animals were subsequently infused with physiological saline except during periods in which the sensitivity of the myometrium to oxytocin was tested. No tests were performed for at least 1 h after the infusion of $PGF_{2\alpha}$ was discontinued and at least 1.5 h were allowed to elapse between tests on any one animal.

Prior to each test uterine activity was recorded for a 20 min control period whilst saline was infused at 1 ml/min. This rate of infusion was used throughout the test period. Oxytocin was then infused at 10 mU/min for 6–10 min depending on the timing of the appearance of contractions. The solution was then changed to contain 10 mU oxytocin/ml in either 10 or 20 m*M* theophylline in physiological saline. After a further 6–10 min the infusion of 10 mU oxytocin/min in the absence of theophylline was recommenced.

Figure 1 demonstrates the effect that was observed in 1 experiment. In all experiments oxytocin increased the frequency and amplitude of uterine contractions. Oxytocin induced uterine activity was reduced by both concentrations of theophylline. In 5 of the experiments both the amplitude and frequency of contraction was affected. On a further 2 occasions the frequency of contractions was relatively unchanged but the amplitude was markedly decreased as in Figure 1. In the remaining 6 experiments the frequency of contractions was affected but not the amplitude.

These findings support the concept that the responsiveness to oxytocin may be related to the generation of cyclic 3′5′ AMP in the uterine muscle cell[4]. It has been demonstrated that the oxytocic effect of $PGF_{2\alpha}$ on the myometrium is also inhibited by theophylline[5]. The doses of theophylline used in the current experiments elevated plasma glucocorticoid secretion under adrenocorticotrophin (ACTH – Synacthen, Ciba) stimulation in the pregnant rabbit (Figure 2). The action of ACTH is thought to involve the generation of cyclic AMP[6], and thus it would appear that the rates of theophylline infusion used in these experiments were at least adequate to raise adrenal cyclic AMP concentrations. The effect of theophylline infusions on myometrial and uterine vein cyclic 3′ 5′ AMP concentrations is currently being investigated.

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*Résumé.* Une lutéolyse ayant été provoquée au moyen de prostaglandine $F_{2\alpha}$ chez la lapine portante, on peut faire apparaître des contractions utérines en administrant de l'ocytocine. Ces contractions sont plus faibles si l'on additionne 10 ou 20 m*M* de théophylline. Cela confirme l'hypothèse d'un rapport entre la sensibilité à l'ocytocine et la production de cyclic 3′,5′-AMP dans la cellule musculaire de l'uterus.

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## Ultrastructure of the Neurohypophysial Glial Cells Following Stalk Transection in the Rat

Despite extensive information on the ultrastructure of neurohypophysial glial cells (pituicytes)[1–12] their exact functional significance is as yet unknown. The following findings on the ultrastructure of these cells under experimental conditions cast some light on their possible function.

*Materials and methods.* Fixation by perfusion with 5% glutaraldehyde of at least 3 rats at 1, 2, 3, 4, 5, 6, 8, 10, 15, 20 and 30 days after hypophysial stalk transection (post trans.) with a Halasz knife[13]. Postfixation in 1% $OsO_4$, embedding in araldite.

*Results and discussion.* Initially, the neurohypophysial glial cells react to the transection of the peptidergic neurosecretory axons by gradually surrounding these axons; at around 5 days post transection practically all axons have been engulfed totally[1–6,14]. Together with the engulfment of the neurosecretory axons an increase in the number of lipid inclusions, lysosomes and glycogen particles is found within the glial cells. The definite hypertrophy of the Golgi apparatus very likely reflects an increase in the synthesis of lysosomal enyzmes[15].

As early as 3 days post trans., crystalloid membrane bounded glial cell inclusions occur (Figure 1). In semithin sections[16] these inclusions are aldehydefuchsin positive which enabled us to trace back their origin to engulfed axons within which crystalloid inclusions are first observed when the neurosecretory granules have fused into a homogeneous substance (Figure 2), after disappearance of their bounding membranes. The latter, together with other axoplasmic constituents are incorporated into dense lamellar bodies (Figure 3). Crystalloid inclusions are found only exceptionally prior the engulfment of the degenerating axons by the glial cells. The formation of these inclusions very likely depends primarily upon lytic enzymes from the glial cells. Following the disappearance of the axolemma phagosomes of varying appearance and size are formed (Figure 3).

At around 8 to 10 days post transection, the disposal of the axons is practically terminated[14], and most of the glial cells are devoid of crystalloid inclusions; subsequently lysosomes and lipid inclusions gradually disappear. Concomitantly the glial cells shrink considerably and agglomerate into epitheloid clusters (Figure 4). Frequently they are interconnected by gap junctions (nexus). 30 days post transection, lipid inclusions are extremely rare and only occasional lysosomes and lipopigments remain (Figures 4 and 5). The mitochondria appear to be more numerous than in control animals. Many glycogen particles are present. The profiles of the rough ER are frequentyl slightly dilated and contain moderately dense material (Figure 5), the Golgi apparatus maintains an active appearance and granulated vesicles may be observed in its vicinity (Figure 6). The general appearance of the glial cells at this stage is reminiscent very much of that of the glial cells in the fetal neurohypophysis prior to the arrival of the neurosecretory axons[17]. The perivascular spaces are wider and contain more collagen fibrils than in control animals, they also have a tendency to invade the spaces previously occupied by the neurosecretory axons; pericytes and fibroblasts are present (Figure 4). A typical inflammatory reaction with occurrence of macrophages is lacking. This, together with the absence of glial scar formation is undoubtedly responsible for the ease with which the denervated neurohypophysis may be invaded again by the regenerating neurosecretory fibres[14].

Whenever neurohypophysial glial cells are not contacted by neurosecretory nerve fibres, they are characterized by the absence of lipid inclusions. This suggests that the latter are related to the process of neurosecretion. It has been proposed previously that they represent a transitory phase of lysosomal activity[15,18–20]. The catabolic activity

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