Table 1. Effect of a) substitution and b) solvent on ring closure of open forms of benzo-1,4-diazepines

a) Substitution (structure **X** → **XII**, in pyridine)

R	R′	R‴	$T^1/_2$*
H	NO_2	H	33 h
H	NO_2	Cl	230 h
H	Cl	H	7 h
Me	Cl	H	4.5 min

b) Solvent effect (structure **XI** → **XIII**)

Solvent:	Water (pH 7)	Pyridine	Dimethoxyethane
$T^1/_2$**:	73 sec	5.5 min	160 min
Chloroform	Dioxan		
280 min	307 min		

*Based on ^{1}H-NMR-measurements. **Based on UV-measurements.

Table 2. Relative rates of formation of diazepam, in vivo*, from latentiated forms

Compound	Molar activity at peak, relative to diazepam	Time of peak activity (min)
Diazepam (**VIII**), R′=Cl, R=Me, R″=H, R‴:	1	immediate
Gly	0.4	15
L-Phe	0.8	2–3
D-Phe	< 0.1	–
L-Leu	0.6	2–3
L-Ala	0.3	2–3
D-Ala	< 0.1	–
L-Lys	1.0	2–3
L-Arg	1.0	2–5
L-Glu	0.5	5–15

*Measured by anti-pentylenetetrazole test for anti-convulsant activity in mice, i.v. administration.

way as latentiated benzodiazepines. The synthesis was achieved by coupling the appropriate 2-aminobenzophenone derivative (**VII**) with a suitably protected and activated dipeptide derivative, followed by deprotection, or by similar attachment of a single amino acid residue to the appropriate glycylaminobenzophenone, as illustrated in the figure below.

The compounds **VIII** with the terminal L-amino acid residues were cleaved by peptidases, in vivo, to release the precursor (**X**) which cyclised at physiological pH to the benzodiazepine; they were also cleaved at a comparable rate in whole blood of various species: rodents, cat, dog, primates and man. The sequence of reactions is illustrated by the diazepam series: pro-drug (**IX**) → open form (**XI**) → diazepam (**XIII**). As expected, compounds with D-terminal amino acids were not cleaved and had no benzodiazepine-like pharmacological activity. The results of the anti-pentylenetetrazole test for anti-convulsant activity in mice, following i.v. administration, were compared with those of parent benzodiazepines for potency (ED_{50}) and time of onset for peak activity.

Measurements using various terminal amino acids combined with the same nucleus established that there were substantial differences in the rate of peptidase cleavage, in vivo. This is illustrated for the diazepam series in which the order for L-amino acids is Phe,Lys,Arg > Leu > Ala > Glu > Gly (table 2). Similar variations were observed for members of other series of latentiated benzodiazepines. These investigations have established that appropriate peptidoaminobenzophenones are novel minor tranquillizers that depend for their pharmacological activity on rapid release of a ring-open precursor of benzodiazepines. Physical properties, such as enhanced solubility in aqueous media compared with the corresponding benzodiazepines and different half-lives, in vivo, provide for novel applications of these compounds.

Ultrastructural changes in dog thyroid follicular cells elicit by concanavalin A in vitro

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Summary. Dog thyroid follicular cells exposed to concanavalin A (Con A) in vitro showed changes in cell shape, induction of colloid droplets and alterations in the distribution of microvilli. Cells exposed to Con A plus suboptimal concentrations of TSH (thyroid stimulating hormone) showed pseudopods and their cytoplasm was virtually occupied with colloid droplets. This findings suggest that Con A potentiated pseudopod and colloid droplet formation induced by TSH.

Recently, Concanavalin A (Con A) has been found to mimic insulin effects on isolated adipocytes[2,3]. It also mimicked the action of a gonad-stimulating peptide in inducing the production of 1-methyladenine in isolated starfish ovary follicle cells[4]. These findings suggest that Con A has the same capacity as a peptide hormone to stimulate certain cell functions. Therefore, one might expect Con A to exert certain TSH-like effects on thyroid follicular cells. Here we report on the effects of Con A on the ultrastructure of dog thyroid follicular cells and on the ultrastructural response to TSH.

Materials and methods. 1. Materials. The compounds used in this study and their sources are listed elsewhere[5]. Con A was purchased from Sigma Chemical Co. (St. Louis, Missouri). 2. Treatment of animals. 12 mongrel dogs, weighing between 16 and 20 kg and previously maintained on a normal iodine, balaced diet and in a controlled environment, were fasted overnight and anesthetized with sodium pentobarbital 50 mg/kg b.wt i.v. These dogs were divided into the following groups: Group A, non-

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suppressed (6 dogs). These dogs were thyroidectomized and the thyroid glands treated as described below. Group B, thyroid powder suppressed (6 dogs). These dogs were given thyroid powder, 300 mg per dog for 3 days, in order to suppress endogenous TSH production. They were operated upon on the fourth day[5]. 3. Preparation of the samples. Immediately after removal, the thyroid lobes were immersed in Earle's solution at 4 °C. They were cut to a thickness of 0.8 mm with a Stadie-Riggs microtome. They were then preincubated in Earle's solution containing 1 mg/ml of both glucose and albumin for 30 min. Slices were transferred directly to individual incubation Erlenmeyer flasks with (treated) or without (controls) the addition of the experimental substances. Preincubation and incubation were carried out in a Dubnoff incubator at 37 °C, shaking at 90 cycles/min. Each flask

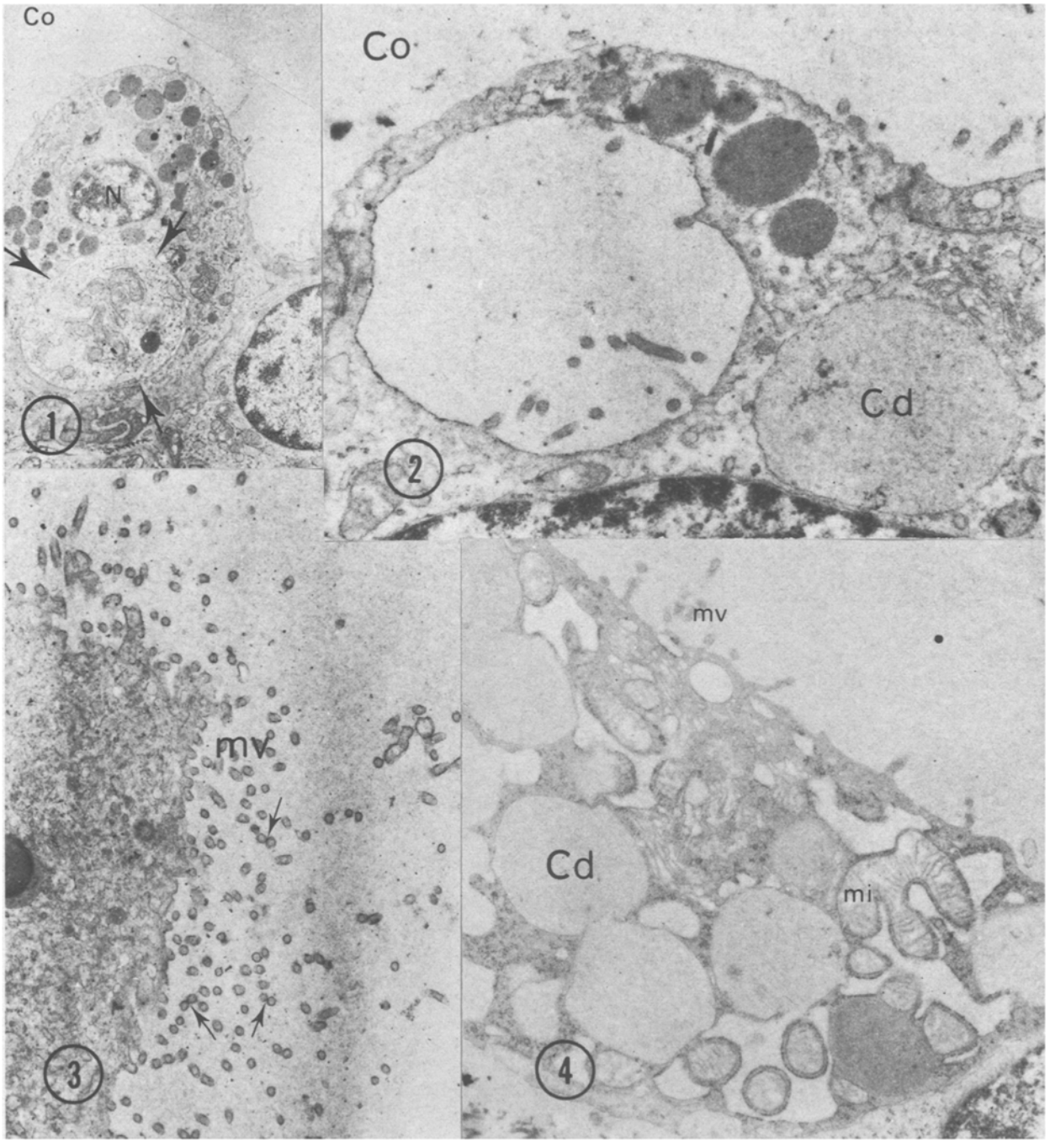

Fig. 1. Exposure of follicular cells to Con A (1500 μg/ml for 180 min) induced a rounding of the apical border. A redistribution of mitochondria is observed. They are limited by and apparently separated from the rest of the cytoplasm by a membrane (arrows) (×6250). Abbreviations used in all figures: Co, colloid; MV, microvilli; N, nucleus; Mi, mitochondria; Cd, colloid droplet.

Fig. 2. Con A (200 μg/ml for 180 min) induced pseudopod and colloid droplet is observed (×21,500).

Fig. 3. Con A (200 μg/ml for 60 min) induced altered distribution of microvilli. Some of them are aggregated in pairs (arrows) (×18,100).

Fig. 4. After exposure of follicular cells to Con A and TSH (200 μg/ml and 0.05 mU/ml, respectively for 180 min), a large increase in colloid droplets, not seen with those concentrations of TSH alone or Con A alone, was observed (×16,000).

contained 2 ml of Earle's solution, 0.5 mg/ml each penicillin G and streptomycin; 1 mg/ml of bovine serum albumin and 1 mg/ml of glucose. Con A and/or TSH were added only to the incubation medium. The flasks were continuously exposed to an atmosphere of 95% O_2 5% CO_2.
4. Histology. Specimens for light and electron microscopy were treated as described elsewhere[5]. Observations were performed with a Siemens Elmiskop IA electron microscope.

Results. 1. Control. a) Basal. Colloid droplets were seen in 10% of cells in group A and they were not observed in group B. b) TSH-stimulated (120-min-incubation). As expected, in response to TSH, 0.1 mU/ml or 2.5 mU/ml, colloid droplet formation was markedly enhanced in both groups A and B. After 2.5 mU/ml, virtually 100% of the cells in both groups A and B had colloid droplets, frequently multiple droplets in a single cell. 2. Effects of Con A-tretment (figures 1–4). a) Without TSH. Cells from both groups A and B exposed to Con A-concentrations from 50 to 2000 µg/ml for 180 min showed ultrastructural changes in a dose dependent manner. Con A-effects were detectable regardless of whether the gland has been suppressed with thyroid powder. In response to Con A, in the concentration range of 50 to 200 µg/ml, pseudopod and colloid droplet formation was observed in the cells after 180 min of incubation (figure 2). About 40% of the cells of both groups A and B showed pseudopods and/or colloid droplets. At 200 µg/ml, Con A also induced, in a small minority of the cells, an altered distribution of microvilli (figure 3). They tended to be agglutinated. These microvilli did not show ultrastructural changes either in the membrane which covers them or in the core of microfilaments. Concentrations in the range of 1000–2000 µg/ml induced a rounding of the apical border in 50% of the cells after a 180-min-exposure to Con A (figure 1). These effects were more commonly seen in small follicles. Pseudopods or colloid droplets were seldom observed with those doses of Con A. A redistribution of mitochondria was observed in some cells when 1000–2000 µg/ml of Con A were used. They were found to be limited by and apparently separated from the rest of the cytoplasm by a membrane (figure 1, arrows). b) With TSH (figure 4). After exposure of follicular cells to both Con A and TSH (200 µg/ml and 0.05 mU/ml, respectively), a large increase in colloid droplets, not seen with those concentrations of TSH alone or Con A alone, was observed. In about 40% of the cells the cytoplasm was virtually occupied by colloid droplets after 90–180 min exposure.

Discussion. This study shows that Con A induce complex changes in the ultrastructure of dog thyroid follicular cells in vitro. The ability of Con A to round up the apical portion of follicular cells (figure 1) is in accordance with the demonstration of a complete rounding of cells in tissue culture induced by exposure to Con A[6]. The clumping of microvilli (figure 3), is also compatible with findings by other investigators. It has been suggested that agglutination of cells by Con A is a function of the presence or absence of microvilli[7]. Probably, microvilli provide large surface that easily come in intimate contact and allow Con A molecules to bind the microvilli of 2 different cells together[7]. In our case, it is possible that a similar phenomenon occurs between microvilli of the same cell.

Con A stimulated the formation of pseudopods and colloid droplets in some cells (figure 2). The droplets seen after incubation with Con A must be newly formed, since they were not observed in control thyroid slices of dogs in which thyroid glands have been suppressed. Con A also potentiated the effects of suboptimal concentrations of TSH (figure 4). These observations suggest that Con A mimicked some of TSH effects.

The present observations must be interpreted as preliminary. However, they show that Con A is capable of altering the ultrastructure of dog thyroid follicular cells in vitro in a dose-dependent manner and of affecting their response to TSH in a characteristic fashion.

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Role of the pituitary in cyproheptadine-induced pancreatic beta-cell toxicity[1]

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Summary. Hypophysectomized rats given cyproheptadine (40 mg/kg) for 10 days exhibited a loss of pancreatic immunoreactive insulin and ultrastructural changes in the cytoplasm of beta-cells. Sham-operated animals given cyproheptadine showed identical changes in pancreatic beta-cells except that cytoplasmic involvement progressed to the formation of large vacuoles. The pituitary is not directly involved with the cyproheptadine-induced depletion of pancreatic insulin but plays a role in the formation of large cytoplasmic vacuoles.

Cyproheptadine is one of several structurally related compounds which can produce unique changes in pancreatic beta cell structure and function in rats[2,3]. These alterations are characterized initially by vesiculation of rough endoplasmic reticulum, a loss of insulin containing secretory granules, and followed later by the formation of large cytoplasmic vacuoles in the beta cells[4]. Decreased pancreatic insulin levels and hyperglycemia were observed in conjunction with these morphologic changes[5]. In a morphologic study, Richardson[6] observed an absence of large vacuoles in the beta cells of hypophysectomized rats administered cyproheptadine for 10 days while sham-operated controls given the drug exhibited typical cyproheptadine-induced changes. Pancreatic insulin content, an important biochemical parameter altered by cyproheptadine administration, was not measured in that study. The present study was performed to determine

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