The Effect of Vitamin D_3 and 25-Hydroxycholecalciferol on Intestinal Transport of Calcium in vivo and in vitro

Following the administration of vitamin D_3, the lag[1,2] is partly due to the time needed for the development of certain biochemical changes in the mucosal cells[3–8], resulting in an increased transfer of calcium. On the other hand, this lag is partly accounted for by the time needed to transform cholecalciferol to more polar metabolites[9–11]. One of them is 25-hydroxycholecalciferol[12,13] which stimulates calcium transport sooner than vitamin D_3[14].

However, this phenomenon has been demonstrated in rats using either in vitro system or indirectly by measuring the activity in blood following the oral administration of labelled calcium. The present experiments were undertaken to examine the development of the effect of vitamin D_3 or 25-hydroxycholecalciferol by a more direct in vivo technique and to compare these results with that of in-vitro test.

Rachitic male albino rats bred in our laboratory were used. The development of vitamin D deficiency was checked by determining fasting serum levels of calcium. A modified in vitro method described by SCHACHTER[15] was used to investigate calcium transport. Calcium absorption was investigated in vivo by ligating the duodenal segment of the small intestine and injecting a known amount of ^{45}Ca labelled $CaCl_2$ into the lumen in the anaesthetized animals[16]. The radioactivity and the total calcium content remaining in the lumen of intestine after 20 min were determined and the rate of absorption was calculated from the decrease of the radioactivity. The variation in the length of the individual loops was also taken into account in the calculation[17]. Data on the amount of total calcium of the lumen were not illustrated because they showed similar pattern to that of the radioactivity measurements. The significance of the results were assessed by Student's *t*-test, the results are given as the mean $\pm$ SD.

Vitamin D_3 (Koch-Light) and 25-hydroxycholecalciferol (kindly supplied from Dr. Kodicek, Dunn Nutr. Lab., Cambridge, England) were administered i.v. 8 or 24 h before the calcium absorption or calcium transport assays.

Under in-vivo conditions, 8 h after the administration of the substances only 25-hydroxycholecalciferol (HCC) exhibited a moderate but significant rise in calcium absorption:

	Vitamin D deficient	100 IU of D_3	100 IU of HCC
Percentual absorption	51.8±8.4 (11)	53.9±13.0 (9)	[a]67.0±9.5 (9)

[a] Statistically significant difference to the untreated rats.

When the period between the injection of the vitamins and the examination of calcium absorption lasted for 24 h, this difference disappeared and calcium absorption was found to be similar either after vitamin D_3 or after 25-hydroxycholecalciferol:

	Vitamin D deficient	50 IU of D_3	50 IU of HCC
Percentual absorption	31.7±11.6 (12)	[a]45.0±9.2 (12)	[a]43.8±8.3 (12)

As present in-vitro results indicate, 100 IU of 25-hydroxycholecalciferol had a more pronounced effect on calcium transport 8 h after its administration than vitamin D_3.

	Vitamin D deficient	100 IU of D_3	100 IU of HCC
S/M value	2.27±0.61 (9)	3.39±0.81 (6)	[a]4.70±0.91 (5)

24 h after the application of the vitamins the increase in calcium transport was similar in both cases:

	Vitamin D deficient	50 IU of D_3	50 IU of HCC
S/M value	2.08±0.42 (6)	[a]3.18±0.48 (5)	[a]3.29±0.54 (6)

[a] Statistically significant difference to the untreated rats.

The methods used to compare the time lag following the administration of 25-hydroxycholecalciferol and vitamin D are frequently indirect ones. In the present experiments, this question was investigated both in vivo and in vitro. According to the results presented, 25-hydroxycholecalciferol stimulates intestinal calcium transport faster than vitamin D_3; however, this difference has disappeared by 24 h. The data obtained by in vivo loop method or everted intestinal sac technique are in a good agreement, suggesting that the capacity of the mucosal cells of transporting calcium was increased due to the treatment. These data are in accordance with the theory that 25-hydroxycholecalciferol exerts more direct effect on the target tissue than vitamin D_3, but do not exclude the possible role of other metabolites in the physiological effect of vitamin D[18–20].

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Zusammenfassung. 8 Stunden nach Injektion von 25-Hydroxycholecalciferol bei rachitischen Ratten steigerte sich der Kalzium-Transport des Duodenum in vivo wie auch in vitro bedeutend. Während dieser Zeit war Vitamin D_3 wirkungslos. 24 h nach der Injektion stimulierten beide Verbindungen in ähnlichem Masse den Kalzium-Transport.

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Mechanoreceptors on the Antenna of the Tobacco Hornworm Moth (*Manduca sexta*)

Although insect mechanoreceptors appear in many diverse forms, the simplest appears to be the sensilla trichoidea. These structures are innervated by a single bipolar neuron with the cell body just under the cuticle. The dendritic portion of this neuronal arrangement generally terminates within the lumen near the base of the sensory hair. In some instances, there may be three neurons terminating in a single hair, one of which dendritically terminates into a disc and is mechanoreceptive, while the other two have a chemosensory function[1].

Numerous functions have been suggested for the sensilla trichoidea, some of which include 1 proprioceptors in the cockroach leg[2]; 2. sound reception in locusts[3]; 3. contact chemoreception in flies[4], and 4. receptors for humidity changes in the tsetse fly[5].

Preliminary studies have indicated a lack of chemoreceptor function for the trichoid sensilla on antennae of the male tobacco hornworm moth, *Manduca sexta*. In view of this information, these sensilla were then investigated for their mechanoreceptive qualities. Only male moths were used for this study because female antennae are relatively void of any large sensory setae.

Method. Isolated head preparations of male tobacco hornworm moths were securely fastened to a paraffin mounting block by means of 2 dissecting needles. The parietal area of the head cuticle was removed and pharyngeal pump muscles cut away. All antennal musculature was severed, leaving only the antennal nerve as the primary connection.

Afferent responses were recorded by means of a hook stainless steel electrode in contact with the antennal nerve. The indifferent electrode was placed in the contralateral eye, while the ground was placed in the head musculature. The antennal nerve between the recording electrode and the deutocerebrum was severed to eliminate any possible efferent activity.

The antenna of the male tobacco hornworm moth is approximately 'key hole' in cross section with 2 rows of hair sensilla on 2 of the 3 sides. A V-shaped fork was formed from fine wire and sized so as to engage these hairs as the fork was moved along the antenna. The tip of the antenna was waxed to a wire loop holder, stationed in the wax mounting block. The antenna was thus maintained in an extended position, permitting the wire form to displace the hairs in selected areas along its length. The fork was attached to a 4 inch high compliance loudspeaker by means of a plastic cross piece cemented to the speaker cone. The apparatus was mounted on a mechanical stage to permit convenient positioning with respect to the moth antenna.

Stimulus was administered by feeding a square wave signal from a Grass S_4 stimulator to the speaker. By adjusting the voltage and time base of the signal, a stimulus having the desired magnitude and duration could be programmed. The signal to the speaker was paralleled to the lower trace of the oscilloscope to provide a stimulus artifact.

The hair sensilla on one antennal segment were displaced distally followed by an equal proximal displacement. The velocity of displacement was 5.4 mm/sec.

The oscilloscope was operated at a sweep speed of 0.2 sec/cm and sensitivity of 1 mV/cm. In some cases, response traces were superimposed to study form and consistency of interspike interval. A 3 min time interval was maintained between successive stimuli in this study.

Antennal segments for histological examination were fixed in situ in cold (4°C), 4% glutaraldehyde solution buffered to a pH 7.4 with s-collidine buffer[6,7]. The tissue was then excised and placed in a beaker containing the cold buffered fixative for 2 to 3 h. After fixation, the material was transferred to s-collidine buffer at 4°C for a period fo 12 h. Dehydration was carried out through solutions of methyl cellusolve, methanol, ethanol, propanol and embedded in 2-hydroxymethylmethacrylate. Sections were cut at 2 µm with a steel knife, mounted and stained with acid fuchsin and methylene blue.

Results (structural). The difference in external morphologies between antennae of male and female hornworm moths is striking (Figure 1). The male antenna has the shape of a key 'hole' in cross section and is aligned with 2 distinct rows of sensilla trichoidea on 2 of the 3 sides. These sensilla form an arc as they meet at their distal extremities. Between these large sensory hairs, additional smaller sensilla (s. chaetica, s. basiconica) are found. The female antenna is generally shorter and circular in cross section. It does not possess any of the larger s. trichoidea noted in the male, but only setae closely appressed to the antennal surface.

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