Sialidase of Transitional Epithelium of Sheep Urinary Tract

Serial assays of sialic acid in transitional epithelium of sheep urinary tract indicated that there was a significant increase of free sialic acid with time of storage at 4°C. It was speculated that these changes could be due to the activity of a sialidase present in transitional epithelium. The experiments performed to test this hypothesis are the basis of this report. It will be shown that sialidase (N-acetylneuraminate glycohydrolase, EC 3.2.1.18) is a lysosomal enzyme of transitional epithelium of sheep urinary tract, with a characteristic acid optimal pH.

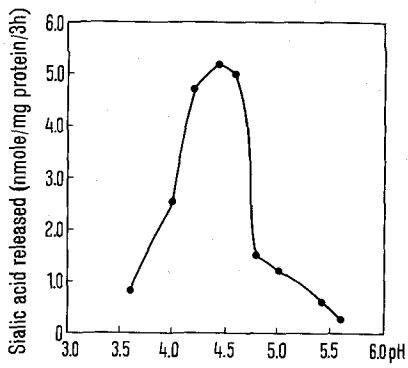
Materials and methods. Transitional epithelium was isolated from sheep urinary bladders[1]. Pellets of exfoliated and washed cells were homogenized with 3 volumes of 0.154 M potassium chloride and centrifuged at 7710 $\times g$ for 1 h. Sialidase activity was determined in the supernatant of the total homogenate, using N-acetylneuraminyl lactose as the substrate[2]. To 0.3 ml of supernatant, 0.1 ml of distilled water containing 0.06 µmole of N-acetylneuraminyl lactose (from bovine colostrum type I. Sigma Chem. Co.) and 0.1 ml of 0.05 M acetate buffer pH 4.4 was added. Incubation was conducted for 3 h at 37°C. At this time, the reaction was stopped by adding 0.1 ml of sodium periodate. Free sialic acid was determined by the thiobarbituric acid procedure[3]. Total proteins were determined by the procedure of LOWRY et al.[4]. For controls, enzyme and substrate blanks were run separately. Sialidase activity was expressed in nmole of sialic acid liberated per mg of protein in 3 h at 37°C.

The optimal pH for activity of the enzyme was determined by incubating the supernatant of the total homogenates in the isotonic solution of potassium chloride with 0.05 M acetate buffer at pHs ranging from 3.6 to 5.6. Maximal activity of sialidase was observed at pH 4.4 (5.1 nmole/mg protein/3 h). Activity was scant or absent at pH over 5.0 (Figure). EDTA at 0.005 M concentration resulted in about 34% of inhibition of enzymatic activity. Calcium ions in the same concentration had no effect. Enzymatic activity was completely inhibited when the samples were preheated in a water bath at 100°C.

For determining the subcellular distribution of sialidase activity, pellets of washed exfoliated cells, homogenized with 5 vol. of 0.25 M sucrose containing 0.001 M EDTA, were subjected to cellular fractionation according to the procedure of DE DUVE et al.[5], as modified by SHETLAR et al.[6]. The subcellular fractions were suspended in 0.1% Triton X-100 and homogenized. The relative purity of fractions was controlled by enzymatic markers (Table I). After an exhaustive dialysis against distilled water, assays of sialidase activity in subcellular fractions were performed at pH 4.4. As shown in Table II, the lysosomal fraction contained highest sialidase activity.

Results and discussion. Our observations indicate that transitional epithelium of urinary tract is a rich source of sialidase. In fact, activity is even higher than in rat lactating mammary gland[7] and mammalian brain[7,8]. The optimal pH of activity is similar to that recorded for chick chorio-allantoic membrane[9], rat liver and rat kidney[10]. Optimal pH is definitely lower than for viral and bacterial sialidases[11]. The intracellular distribution and the distinct acid pH optimum indicate that it is a lysosomal enzyme.

Transitional epithelium, the cellular lining of the urinary tract organ, contains high levels of certain carbohydrates, such as sialic acid, which are concentrated in the micro-



Effect of pH on sheep transitional epithelium sialidase.

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Table I. Distribution of enzymatic activity in subcellular fractions of sheep transitional epithelium[a]

	Succinic dehydrogenase	B-Glucuronidase	Glucose-6-Phosphatase
Nuclei	6.4	7.8	12.0
Mitochondria	57.4	10.2	12.8
Lysosomes	16.8	48.0	8.8
Microsomes	13.0	19.6	62.0
Soluble fraction	6.4	14.4	4.4

[a] Results are expressed in percent of total activity.

Table II. Subcellular distribution of sialidase in sheep transitional epithelium

Fraction	Sialic acid released (nmole/mg protein/3 h)
Nuclear	0.82
Mitochondrial	6.53
Lysosomal	30.42
Microsomal	6.99
Supernatant	1.40

somal fraction and mainly in plasma membrane[12]. Corresponding with these observations, a surface mucous coat or glycocalyx and numerous lysosomes have been observed in this epithelium. Since it has been postulated that the Tamm-Horsfall urinary sialoglycoprotein is related to urinary tract cell surface[13], the notably high content of sialic acid and sialidase in sheep transitional epithelium might be coincidental findings of some biological significance. In fact, studies in course indicate that rat transitional epithelium contains low levels of sialic acid and negligeable sialidase activity[14].

Resumen. El epitelio de transición de cordero contiene actividad de sialidasa. Se demuestra que esta enzima se encuentra en lisosomas de este tejido y posee el pH óptimo ácido característico de enzimas lisosomales.

A. CANDIOTTI, N. IBAÑEZ and B. MONIS

Instituto de Biología Celular, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Casilla Postal 362, Córdoba (Argentina), 1 November 1971.

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Aggressive Behaviour Induced by Marihuana Compounds and Amphetamine in Rats Previously Made Dependent on Morphine

It has been described recently that rats, previously made physically dependent on opioids, show a traumatic aggressive behaviour when treated with amphetamine during the withdrawal phase[1,2]. The description of the fighting behaviour showed by these rats was similar to that of starved rats treated chronically with marihuana[3,4]. The present work was carried out to observe whether cannabis would induce aggressive behaviour in non-starved rats during withdrawal from previous morphine administration, and to compare the aggressive behaviour induced by these drugs.

Material and methods. Animals and Drugs. 124 male 3-month-old Wistar rats were maintained at 23 ± 1°C in air conditioned laboratories. A standard food pellet diet and tap water were provided ad libitum. Morphine hydrochloride (E. Merck, Darmstadt), D-amphetamine sulfate (Sigma Chemical Company) and (−) Δ^9-*trans*-tetrahydrocannabinol (THC) obtained from United Nations were employed. Morphine and amphetamine were dissolved in 0.9% NaCl solution; THC was suspended as recommended by others[5]. The amounts used are expressed in terms of the salts. Three experiments were carried out (see Table).

Experiment 1. 30 rats were housed in pairs in wire cages measuring $16 \times 30 \times 18$ cm. They received daily 3 i.p. injections of morphine, at 5 h intervals; the initial dosage was 12 mg/kg/day (3 injections of 4 mg/kg) which was doubled every 2nd day up to 96 mg/kg/day. 24, 48 and 72 h after the last morphine injection, therefore during withdrawal phase, 5 pairs of rats received 1.0 ml/kg of saline (group I), 5 pairs received 5.0 mg/kg of THC (group II) and the remaining 5 pairs 2.0 mg/kg of D-amphetamine (group III). The injections were i.p. and the pairs of rats were observed following the next 12 h for signs of depression, aggressiveness and stereotypy. 24 h later the animals were observed again. Depression was defined by the lack of motion and/or laying down of the rats; aggressiveness, scored in seconds, was the time the animals remained in a standing position trying to bite each other[3], and stereotypy was defined by sniffing, licking or biting the cage wire netting.

Experiment 2. In a first phase, 30 pairs of rats were treated with morphine as in experiment 1; however, the treatment was prolonged up to the dosage of 768 mg/kg/day. 8 other pairs of control rats were similarly injected with saline. At 24 and 48 h of withdrawal, 6 pairs received saline (group I), 8 pairs received 5.0 mg/kg of THC, 8 pairs received 10 mg/kg of a marihuana extract and the last 8 pairs were injected with 2.0 mg/kg of D-amphetamine (group III). As the results with THC and marihuana were similar, these 16 pairs of animals were considered together as group II. All injections were i.p. and the rats were observed as in experiment 1. Each 2 pairs of the control group received the same treatment given to groups I to III. As these animals showed only the typical effects of the drugs, they will not be included in the Table. In a second phase, starting 24 h after the last treatment with THC and amphetamine, morphine administration (and saline for control group) was reinitiated for all animals; now, 2 doses of 200 mg/kg of morphine were given daily, for 6 more days; at 48 and 72 h of a second withdrawal period the rats were submitted to same treatment as above.

Experiment 3. 18 rats were housed individually in the wire cages. Morphine addiction was induced by initial i.p. injections followed later by oral route according to a modification of the procedure by NICHOLS[6]. Injections of morphine were given twice daily at 12 h intervals; the initial dose was 10 mg/kg/day (2 daily injections of 5 mg/kg) and was doubled every 3rd day up to 160 mg/kg/day; the animals remained 3 more days on this dosage. Next, the animals were put for 6 weeks in a 3-day schedule of oral administration of liquids as follows: 1st day with water ad libitum, 2nd day without any liquid, 3rd day with morphine 0.8 mg/ml; the average intake of morphine in this phase was 141 ± 51 mg/kg/day (average $\pm$SD). 2 choice-tests between water and 0.8 mg/ml of morphine[6], at days 21 and 42, revealed that the animals preferred to drink morphine. After the second choice-test, animals were allowed to drink only the 0.8 mg/ml mor-

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