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Substrate stimulation of organic anion transport in newborn dog kidney and choroid plexus*

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THE KIDNEY in the newborn of several species is immature both anatomically[1,2] and functionally.[3] Glomerular filtration rate, renal handling of electrolytes, concentrating and diluting mechanisms and transport of a variety of organic compounds are less in the newborn than in the adult.[3]

Accumulation of *p*-aminohippuric acid (PAH) by renal cortical slices from newborn animals was less than that by slices from adults.[4,5] Treatment of young rats and rabbits with substrates of the organic acid transport system (i.e. penicillin or PAH) enhanced PAH accumulation by renal cortical slices from these animals.[6,7] This stimulation was specific for organic acid transport since base transport, measured by *N*-methylnicotinamide (NMN) accumulation, was unchanged by treatment. Stimulation of PAH accumulation by penicillin appeared to be associated with protein synthesis, perhaps specific transport enzymes.[8,9]

The choroid plexus has been shown *in vitro* to actively accumulate a wide variety of molecules including organic anions.[10–12] The process of choroid plexus transport is believed to be similar to that in renal tubules. Also like the kidney, transport of organic anions by the choroid plexus is immature in the newborn.[11,12] It was reasoned that accumulation of PAH by choroid plexus might be stimulated by penicillin treatment as it is in the kidney.

The purpose of this investigation was to determine the effect of substrate (i.e. penicillin) treatment on organic acid transport in newborn dog kidney and choroid plexus.

Mongrel pups were allowed to stay with the bitch until used for an experiment. Procaine Penicillin G suspension (Duracillin; Eli Lilly & Co., Indianapolis, Ind.) was administered i.m. or s.c. in a dose of 300,000 IU/kg twice daily for 3 days prior to 1 or 2 weeks of age. Control littermates were injected with physiological saline (pH 7·4). Forty-eight hr after the last injection, pups were weighed and killed by decapitation.

The kidneys were rapidly removed, freed of capsule, weighed and placed in iced Ringer-phosphate solution. Renal cortical slices 0·3–0·4 mm thick were prepared freehand. The outer single slices were pooled, incubated together and termed outer renal cortex; deeper slices (second and inward) were pooled and termed inner renal cortex. Duplicate samples of approximately 100 mg of slices were incubated in 2·7 ml of Cross and Taggart phosphate medium[13] in a Dubnoff metabolic shaker for 90 min at 25° under 100% oxygen. The medium contained $7{\cdot}4 \times 10^{-5}$ M PAH and $6{\cdot}0 \times 10^{-6}$ M NMN-^{14}C (4·6 mCi/m-mole). After incubation, slices were quickly removed from the medium, blotted, weighed and analyzed for PAH and NMN as reported previously.[14]

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TABLE 1. PAH AND NMN ACCUMULATION BY OUTER AND INNER RENAL CORTICAL SLICES AND PAH ACCUMULATION BY THE LATERAL VENTRICULAR (LVCP) AND FOURTH VENTRICULAR (FOURTH CP) CHOROID PLEXUSES FROM A LITTER OF 1-WEEK-OLD MONGREL PUPPIES

Animal No.	Treatment 1*	PAH T:M ratio†		NMN T:M ratio†		PAH T:M ratio‡	
		Outer cortex	Inner cortex	Outer cortex	Inner cortex	LVCP	Fourth CP
1	Control	1·97	4·07	1·95	2·24	2·18	1·99
2	Penicillin	3·23	8·35	1·25	2·47	1·37	2·60
3	Penicillin	4·20	7·30	2·05	2·27	3·98	2·59
4	Control	2·23	4·31	1·23	2·09	2·86	2·04
5	Control	3·45	6·15	1·35	2·29	2·72	1·74
6	Penicillin	3·41	6·79	1·52	2·91	2·88	2·39
7	Penicillin	3·90	7·54	1·27	2·14	3·08	4·15
Litter values (mean ± S. E.)	Control	2·55 ± 0·46	4·84 ± 0·66	1·51 ± 0·22	2·21 ± 0·06	2·59 ± 0·21	1·92 ± 0·09
	Penicillin	3·69 ± 0·22	7·50 ± 0·32	1·52 ± 0·19	2·45 ± 0·17	2·83 ± 0·54	2·94 ± 0·41

* Control animals were injected with saline (1 cm^3/kg, b.i.d. for 3 days); penicillin-treated animals were injected with Procaine Penicillin G (300,000 IU, b.i.d. for 3 days).

† Measurements were made at 25° under 100% O_2 for 90 min.

‡ Measurements were made at 23° under 95% O_2–5% CO_2 for 60 min.

TABLE 2. EFFECT OF PENICILLIN TREATMENT ON PAH AND NMN ACCUMULATION BY THE KIDNEY OF 1- AND 2-WEEK PUPPIES

Group*	Age (weeks)	n† (litters)	Kidney T:M ratio‡			
			Outer cortex		Inner cortex	
			PAH	NMN	PAH	NMN
Control	1	5	2·85§	1·38	4·46	1·84
Penicillin	1	5	3·47	1·35	5·37	1·85
Difference (± S. E.)			0·62 ± 0·55	0·03 ± 0·03	0·91 ± 0·50	0·01 ± 0·03
Control	2	5	4·36	1·91	5·19	2·25
Penicillin	2	5	5·10	1·71	6·44	1·99
Difference (± S. E.)			0·74 ± 0·22¦	0·20 ± 0·14	1·25 ± 0·40‖	0·26 ± 0·13

* Control animals were injected with saline and penicillin animals with Procaine Penicillin G.
† A total of 30 puppies at 1 week and 30 puppies at 2 weeks were used.
‡ Ratios were determined after incubation for 90 min at 25° under 100% oxygen.
§ A mean value for each animal was calculated from duplicate determinations. Using these, an average value per litter (for control and penicillin-treated animals, separately) was calculated: The group mean reported is a mean with S. E. calculated from litter average values.
¦ Difference between control and treated animals is significant ($P < 0·05$); paired comparison.

TABLE 3. EFFECT OF PENICILLIN TREATMENT ON PAH ACCUMULATION BY THE CHOROID PLEXUS OF 1- AND 2-WEEK PUPPIES

Group*	Age (weeks)	n† (litters)	PAH T:M ratio‡	
			Lateral choroid plexus	Fourth choroid plexus
Control	1	4	2·81§	1·95
Penicillin	1	4	3·31	2·53
Difference (± S. E.)			0·50 ± 0·14¦	0·58 ± 0·19‖
Control	2	3	3·56	3·25
Penicillin	2	3	3·62	3·20
Difference (± S. E.)			0·06 ± 0·23	0·05 ± 0·17

* Control animals were injected with saline, and penicillin-treated animals with Procaine Penicillin G.
† A total of 24 puppies at 1 week and 20 puppies at 2 weeks were used.
‡ Ratios were determined after incubation for 60 min at 23° in 95% O_2 5% CO_2 atmosphere.
§ An average value per litter (for control and penicillin-treated animals, separately) was calculated. The group mean reported is a mean with S. E. calculated from litter averages.
‖ Difference between control and treated animals is significant ($P < 0·05$); paired comparison.

The brain was removed and both lateral and fourth ventricular choroid plexuses were excized and placed in 3 ml of incubation medium. The medium, an artificial cerebrospinal fluid,[11] had trace amounts of PAH (Glycyl 2-^{3}H; 2 μCi/ml). The medium was equilibrated with 95% O_2–5% CO_2 for 30 min prior to and throughout the incubation period. Tissue was incubated at 23° in a Dubnoff metabolic shaker for 1 hr, removed, blotted, weighed and digested in 0·5 ml of 25% KOH. Duplicate 100-μl samples of digested tissue or incubation fluid were analyzed for PAH as reported previously.[11]

Accumulation of PAH by kidney was expressed as the tissue to medium (T:M) ratio which represents the concentration of PAH/g of tissue (wet wt) divided by the concentration of PAH/ml of medium. For NMN-^{14}C (kidney) or PAH-^{3}H (choroid plexus), the T:M ratio represents dis/min per g of tissue (wet wt) divided by the dis/min per ml of medium.

The data from all treated animals in a litter were averaged as were the data from control littermates. These averages were used for statistical analysis. Data were analyzed statistically using Student's *t*-test, paired and unpaired comparison.[15] The 0·05 level of probability was used as the criterion of significance.

The ability of renal cortical slices to accumulate organic anions is low in tissue from newborn dogs and increases with age.[4,5] Furthermore, the degree of maturity of the organic anion transport system in newborn kidneys is related to the depth (from the capsule) of the renal cortical slice.[4] Accumulation of PAH by renal cortical slices from young rats and rabbits is significantly enhanced by treatment with substrates of the organic acid transport system,[6–8] suggesting that one stimulus to normal development is an increasing functional load.

PAH accumulation in renal cortical slices and choroid plexuses and NMN accumulation in renal cortical slices were measured in littermates treated with penicillin or saline. Results from one litter are presented in Table 1. Increased maturity of the transport systems in the inner cortical slices is apparent since in every animal accumulation of PAH and NMN was greater by inner cortical slices. Similarly, uptake by the lateral ventricular choroid plexus was greater than that observed in the fourth ventricular choroid plexus in five of the seven animals. Penicillin treatment increased PAH accumulation by inner and outer renal cortical slices and lateral and fourth ventricular choroid plexuses in this litter of animals.

Differences in maturity of transport by the kidney in control animals are indicated by data summarized in Table 2. The PAH T:M ratio for outer renal slices from 1-week control animals was 2·85 ± 0·55 while that at 2 weeks was 4·36 ± 0·75. Similarly, the T:M ratio for inner renal slices was 4·46 ± 0·68 at 1 week and 5·19 ± 0·86 at 2 weeks. NMN accumulation in outer and inner renal cortical slices from control animals was significantly increased at 2 weeks, although the maximum T:M ratios were considerably less than those for PAH (outer cortex, 1·38 ± 0·13 at 1 week, 1·91 ± 0·18 at 2 weeks; inner cortex, 1·84 ± 0·16 and 2·25 ± 0·13 for 1 and 2 weeks respectively). These differences with age and depth of cortex are similar to those reported by Rennick *et al.*[4]

Treatment of puppies with penicillin produced an increase in the accumulation of PAH by outer and inner renal cortical slices in four out of five litters at both 1 week and 2 weeks of age. The difference between values for control and penicillin-treated 2-week animals was statistically significant for both outer and inner cortex (Table 2); the difference was not significant for 1-week-old animals. The lack of statistical significance at 1 week of age may be explained in part by the greater variability in these animals when compared to the 2-week ones (Table 2).

Even though PAH accumulation in outer and inner renal cortical slices was higher in penicillin-treated animals, the kidney and body weights in these animals were not different from controls. There was no significant effect of penicillin treatment on organic base transport (NMN) in outer or inner renal cortical slices from either age group (Table 2).

Accumulation of PAH by lateral and fourth ventricular choroid plexuses from 2-week-old puppies was greater than that in 1-week-old puppies in both the control and penicillin-treated animals (Table 3). The PAH T:M ratio for the lateral ventricular choroid plexus (LVCP) from 1-week control animals was 2·81 ± 0·26 and 3·56 ± 0·28 at 2 weeks and for the fourth ventricular choroid plexus (fourth CP) at 1 week was 1·95 ± 0·03 and 3·25 ± 0·17 at 2 weeks. Investigators have previously reported an increase in organic anion accumulation in pups from 1 to 2 weeks of age[11,12] and the lower accumulation of the fourth CP, when compared with LVCP, confirms data of Bierer and Heisey.[11]

The T:M for the LVCP was increased significantly by penicillin administration in all four 1-week-old litters but in only one out of three litters at 2 weeks of age. PAH accumulation by the fourth CP was enhanced by penicillin in three out of four litters at 1 week of age but in only one out of three litters at 2 weeks. The effect at 1 week of age was statistically significant but not at 2 weeks of age (Table 3). The wet weight of the brain, LVCP and fourth CP was not significantly different in control and treated animals.

These data extend the observation of substrate stimulation of the organic anion transport system in kidneys to a third species, the dog. Since treatment of puppies with penicillin produced an increase in PAH accumulation by renal cortical slices and choroid plexus, the data suggest that anion transport in these tissues is similar and that the functional capacity of these transport systems may normally develop in response to an increased load. Furthermore, the different age at which stimulation occurs in these two tissues suggests that they develop at different rates.

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Potentiating effect of cannabidiol on Δ^9-tetrahydrocannabinol-induced changes in hepatic enzymes

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IN RECENT years, several observations have been made indicating that the pharmacological and behavioural effects of Δ^9-tetrahydrocannabinol (Δ^9-THC), the principle active component of cannabis,[1–3] are influenced to a great extent by the other major components e.g. cannabidiol, cannabinol etc. present in the cannabis preparations.[4–6] Karniol and Carlini[6] observed that Δ^9-THC content by itself did not explain the biological activity of different Brazilian cannabis samples. Previous reports made from this laboratory[7,8] indicated that using the same dosages in terms of Δ^9-THC content, cannabis extract was in all cases found to be more active in biochemical response than pure Δ^9-THC. The present report deals with the effect of cannabidiol (CBD) on Δ^9-THC-induced activities of two hepatic enzymes, tyrosine α-keto glutarate transaminase (TKT) and tryptophan pyrrolase (TPO), of rats.

In the present experiment, the pure Δ^9-THC and cannabidiol (CBD) used, were provided by the United Nations Narcotics Laboratory, Geneva and the National Institute of Mental Health, Washington, U.S.A., respectively.

Four groups of adult male albino rats weighing about 100–120 g were used in this experiment. The first group was injected intraperitoneally (i.p.) with the suspension of pure Δ^9-THC at doses 5 mg/kg and 25 mg/kg, the second group with the suspension of pure CBD at doses 2 mg/kg and 10 mg/kg and the third group with Δ^9-THC and CBD simultaneously at doses 5 mg/kg + 2 mg/kg and 25 mg/kg + 10 mg/kg respectively. The control group received the saline tween vehicle in equivalent volume by the same route. Activities of tyrosine α-ketoglutarate transaminase (TKT) and tryptophan pyrrolase (TPO) and the protein concentration were determined as previously described.[7]