preparation occurred to about the same extent in the presence and absence of added Na^+ DeFeudis et al.[3].
In sum, it seems apparent that physiological media can be used in further studies aimed at determining the binding of 'active' amino acids to their synaptic receptors. Such studies can be conducted with crude synaptosomal-mitochondrial preparations, as well as with purified membrane preparations, using 15-min incubation at 0 °C.

Compartmentation of GABA, glycine and β-alanine in regions of rat CNS

Parameter	Value*	Total amino acid pool (%)	References
GABA, cerebral cortex			
Total pool	2 μmoles/g	100	18-21
Na^+-dependent binding**	215 nmoles/g	11	22, 23
BMI-displaceable binding***	60 pmoles/g	0.003	1, 24
Glycine, spinal cord			
Total pool	4 μmoles/g	100	25
Na^+-dependent binding**	190 nmoles/g	4.8	22
Strychnine-displaceable binding***	160 pmoles/g	0.004	2, 3
β-Alanine, brain stem-plus-spinal cord			
Total pool	75 nmoles/g	100	26
Na^+-dependent binding**	75 nmoles/g	100	27
Strychnine-displaceable binding***	45 pmoles/g	0.06	4

* All values are expressed per g original wet wt of tissue. ** Values are for maximal binding capacities (B_{max}). *** For GABA, this value represents the amount displaced by 10^{-3}M bicuculline-methiodide (BMI); for glycine and β-alanine, these values represent the amounts displaced by 10^{-3}M strychnine-SO_4.
Note: Dissociation constants for antagonist-sensitive 'high-affinity' binding components were 5×10^{-8}M for GABA[1] and β-alanine[4] and 1.8×10^{-7} M for glycine[3].

1 F.V. DeFeudis and E. Somoza, Gen. Pharmac. *8*, 181 (1977).
2 F.V. DeFeudis, J. Fando and L.M. Orensanz Muñoz, Experientia *33*, 1068 (1977).
3 F.V. DeFeudis, L.M. Orensanz Muñoz and J. Fando, Gen. Pharmac., *9*, 171 (1978).
4 F.V. DeFeudis, L.M. Orensanz Muñoz and J. Fando, Gen. Pharmac. *8*, 311 (1978).
5 F.V. DeFeudis, Progr. Neurobiol. *9*, 123 (1977).
6 F.V. DeFeudis, Acta physiol. latinoamer., *27*, 131 (1978).
7 J.S. DeBelleroche and H.F. Bradford, Progr. Neurobiol. *1*, 275 (1973).
8 K. Sano and E. Roberts, Biochem. Pharmac. *12*, 489 (1963).
9 S.R. Zukin, A.B. Young and S.H. Snyder, Proc. natl Acad. Sci., USA *71*, 4802 (1974).
10 S. Fiszer de Plazas and E. DeRobertis, J. Neurochem. *25*, 547 (1975).
11 S.J. Enna and S.H. Snyder, Brain Res. *100*, 81 (1975).
12 F. Valdés and F. Orrego, Brain Res. *97*, 277 (1975).
13 F. Valdés, C. Muñoz, A. Feria-Velasco and F. Orrego, Brain Res. *122*, 95 (1977).
14 W.E. Müller and S.H. Snyder, Brain Res. *143*, 487 (1978).
15 S.J. Enna, M.J. Kuhar and S.H. Snyder, Brain Res. *93*, 168 (1975).
16 S.J. Enna, H.I. Yamamura and S.H. Snyder, Brain Res. *101*, 177 (1976).
17 A.B. Young and S.H. Snyder, Molec. Pharmac. *10*, 790 (1974).
18 S. Berl and H. Waelsch, J. Neurochem. *3*, 161 (1958).
19 Y. Nagata, Y. Yokoi and Y. Tsukada, J. Neurochem. *13*, 1421 (1966).
20 F.V. DeFeudis and K.A.C. Elliott, Can. J. Physiol. Pharmac. *46*, 803 (1968).
21 Y. Yoshino, F.V. DeFeudis and K.A.C. Elliott, Can. J. Biochem. *48*, 147 (1970).
22 F.V. DeFeudis and N. Schiff, Expl Neurol. *48*, 325 (1975).
23 E. Somoza, M.P. Pugnaire, L.M. Orensanz Muñoz, C. Gonzalez Portal, A. Elena Ibañez and F.V. DeFeudis, J. Neurochem. *28*, 1197 (1977).
24 F.V. DeFeudis, G. Balfagón, M.R. de Sagarra, P. Madtes, E. Somoza and J. Gervas-Camacho, Expl Neurol. *49*, 497 (1975).
25 P.J. Roberts and P. Keen, Brain Res. *74*, 333 (1974).
26 R. Martin del Rio, L.M. Orensanz Muñoz and F.V. DeFeudis, Expl Brain Res. *28*, 225 (1977).
27 L.M. Orensanz Muñoz, F.V. DeFeudis and J. Fando, Gen. Pharmac. *8*, 325 (1977).

The influence of pH on the sex-related differences in renal organic ion transport[1]

A. Small, L.D. Homer and R.S. Ide

Department of Experimental Medicine, Naval Medical Research Institute, Bethesda (Maryland 20014, USA), 29 November 1977

Summary. The stimulating effects of elevated medium pH and androgen on in vitro transport of p-aminohippurate and N-methylnicotinamide (NMN) were additive, although the androgenic effect was pH-dependent only in the case of NMN. The similarity of response of the 2 systems supports the idea of a common passive efflux pathway for organic anions and cations.

The accumulation of both organic anions and cations has been shown previously to be greater in renal cortical slices from male rats than in slices from females[2], corresponding to differences that exist in vivo, and thought to be due to the action of androgenic hormone. Although it is also known that in vitro transport of these ions is pH dependent, there has been no study of the influence of pH on the sex-related differences. The purpose of this study was to investigate the interactions between pH and androgen on the uptake of p-aminohippurate (PAH) and N-methylnicotinamide (NMN) because of the information that such interactions might provide about the transport mechanisms.
Materials and methods. Cortical slices were prepared from kidneys removed from ether-anesthetized Sprague-Dawley rats, and approximately 150 mg tissue was incubated in 3 ml of Cross and Taggart phosphate buffer medium[3] that included acetate (10^{-2} M), PAH (10^{-4} M) and ^{14}C-NMN ($6\cdot10^{-5}$ M; New England Nuclear Corp., Boston, MA).

Initial buffer pH (range: 6.0–8.0) was established by the ratio of Na_2HPO_4/NaH_2PO_4 and ascertained by direct measurement. Following a 90 min incubation at 25 °C under 100% O_2, the amount of PAH and NMN in tissue and buffer medium was measured using methods described previously[4], and accumulation was expressed as the slice: medium concentration ratio (S/M). The pH of incubation was considered to be the final medium pH, since small pH changes occurred during incubation.

Results. Figure 1 shows the relationship between final buffer pH and the uptake of PAH into kidney slices from male and female rats. The pattern for either sex is similar to that reported previously; S/M was significantly higher when incubation medium pH was more alkaline ($p < 0.01$; analysis of variance). The same analysis showed that there was a consistent, significant difference in uptake between sexes throughout the pH range studied. Moreover, there was no correlation between pH and the magnitude of the sex-related difference (△S/M-PAH), indicating that the difference between sexes was approximately the same at all pH values. Figure 2 shows the uptake of NMN as a function of pH. As in the case of PAH, for slices from both males and females, S/M-NMN increased as incubation pH was raised ($p < 0.01$), and there was a consistent difference between sexes throughout the pH range ($p < 0.01$). However, in contrast with results for PAH, there was a significant, positive correlation between the magnitude of the androgenic effect and final incubation pH.

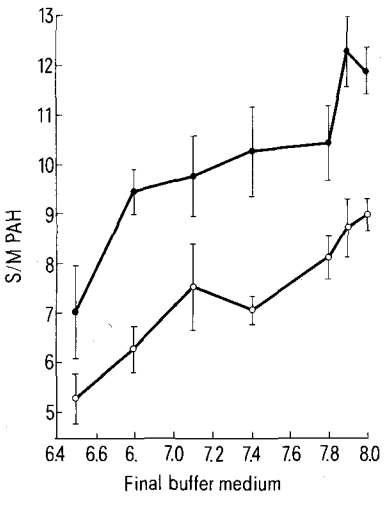

Fig. 1. Effect of buffer pH on steady-state accumulation of p-aminohippurate (PAH) by slices of rat kidney cortex. Accumulation is expressed as the slice: medium ratio (S/M). pH is the post-incubation value. ● are data for male animals; ○ represent females. Each point represents the mean±SEM from 6 animals, except in the case of the values for pH 8.0; the latter represents data from 24 animals, pooled from the 4 groups that had a final pH of 8.0.

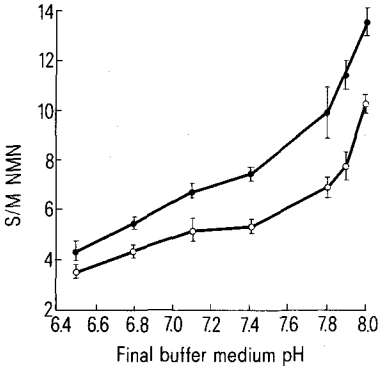

Fig. 2. Effect of buffer pH on steady-state accumulation of N-methylnicotinamide (NMN) by slices of rat kidney cortex. Presentation of data is the same as in figure 1.

Discussion. There is abundant evidence that testosterone enhances the transport of both organic anions and cations, even though the transport of anionic species like PAH is mediated by a specific system which is different than the one that transports cations like NMN[5,6]. The results of the present study confirm earlier observations of the pH dependence of both PAH and NMN uptake[7], although it was not possible to demonstrate a pH optimum at or slightly above pH 8.0 since that value was the upper limit, imposed by the buffer system, in the present experiments. Kinetic studies[2] have shown that androgenic stimulation of PAH uptake into renal slices is associated with a faster rate of influx and slower efflux rate. On the other hand, separate studies[7] indicated that elevated pH did not accelerate influx, and thus may enhance PAH accumulation by decreasing efflux from the slice, or by increasing some form of PAH trapping. The data presented here indicate that the effects of pH and those of androgen are superimposable; therefore, if a decreased rate of PAH efflux is partly responsible for the augmented S/M ratio seen after either testosterone or elevation of incubation pH, then the additive response to these 2 conditions suggests that neither produces a maximal change in PAH efflux rate, and possibly, that more than 1 mechanism is involved in decreasing the rate of efflux. Aside from transport specificity, other characteristics of the system that transports NMN are different than those for the system that is specific for organic anions[8]. The data presented here demonstrate yet another difference between the 2 systems: the effect of androgen on NMN uptake is dependent on incubation pH, whereas its effect on PAH transport is not. In spite of the noteworthy differences between the transport systems, there are also well known similarities between them, particularly their apparent co-location in proximal tubular cells. The results of the present work, indicate additional, marked similarities in the response of both systems to the dual influences of pH and androgen. Although these 2 systems are noncompeting, it is tempting to speculate that transport of both families of molecules is partly determined by a common cellular characteristic, such as passive efflux of the transported species.

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2 H.M. Bowman and J.B. Hook, Proc. Soc. exp. Biol. Med. *141*, 258 (1972).
3 R.J. Cross and J.V. Taggart, Am. J. Physiol. *161*, 181 (1950).
4 A. Small and R.S. Ide, Cryobiology *13*, 328 (1976).
5 L.I. Kleinman, M.S. Loewenstein and L. Goldstein, Endocrinology *78*, 403 (1966).
6 D.M. Ferguson and B.F. Matthews, J. Physiol. *169*, 24P (1963).
7 H.M. Bowman, G.H. Hirsch and J.B. Hook, Experientia *29*, 955 (1973).
8 A. Farah, M. Frazer and E. Porter, J. Pharmac. exp. Ther. *126*, 202 (1959).