marrow material, one should keep in mind that sometimes differences between bones may be due only to the different marrow content. The exact physiological meaning of the differences presented in the marrow component of the bone in male and female rats is yet to be ascertained. However, these data may be very useful in assessing skeletal changes induced by osteoporosis, since rat and particularly its femoral bone are among the most often studied objects in this field.

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Can the binding of GABA, glycine and β-alanine to synaptic receptors be determined in the presence of a physiological concentration of Na^+?

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Summary. Bicuculline- and strychnine-sensitive components of the binding of GABA, glycine and β-alanine, which can be demonstrated in the presence of a physiological concentration of Na^+, might be related to synaptic receptors.

Transmitter-candidate amino acids are taken up by CNS fractions in vitro by potent Na^+-dependent binding and transport mechanisms which involve pre-synaptic elements and glia. Thus, one may believe that the binding of these amino acids to their post-synaptic receptors cannot be determined in the presence of physiological concentrations of Na^+. However, recent studies have revealed that such interactions can be determined under physiological conditions if studies are conducted at 0 °C (to suppress active transport mechanisms), if accurate corrections are applied to the data, if the particles are sufficiently depleted of their contents of endogenous amino acids, and if low concentrations (10^{-10}–10^{-7} M) of the labelled active ligands are employed.

Representative data from our recent publications on the binding of GABA[1], glycine[2,3] and β-alanine[4] will be used here to show components which are sensitive to in vivo antagonists of the depressant actions of these amino acids and which might be related to synaptic receptors. However, it should be realized that the antagonists used, strychnine and bicuculline-methiodide (BMI), while being the most reliable ones available today, possess many nonspecific actions[5,6].

The methods used in these experiments have been discussed in detail[1–6]. In all cases, 'synaptosomal-mitochondrial' (P_2) fractions of rat CNS regions, which are known to contain post-synaptic thickenings and post-synaptic membranes[7], were incubated with labelled ligands for 10–15 min at 0 °C. Many studies have indicated that essentially maximal values for binding of these amino acids to cerebral subcellular particles are obtained under these conditions[8–14]. All operations were performed at 0 °C, rather than at higher temperatures, to prevent active transport and catabolism of the amino acids and tissue autolysis.

The table provides values for the compartmentation of GABA in cerebral cortex, glycine in spinal cord, and β-alanine in spinal cord-plus-brain stem, in terms of total tissue content, total binding in the presence of a physiological concentration of Na^+ (i.e., Na^+-dependent binding which occurred mainly to carrier-transport sites) and antagonist-sensitive binding in the presence of Na^+ (i.e., binding to presumed synaptic receptor sites). It is evident that synaptic receptor compartments for these 3 inhibitory amino acids are quite similar at about 45–160 pmoles amino acid/g original wet wt of tissue. Also, Na^+-dependent binding and BMI-displaceable binding of GABA, as percentages of the total GABA present in cerebral cortex (at 11% and 0.003%, respectively) are quite similar to corresponding values calculated for glycine in spinal cord (4.8% and 0.004%, respectively). However, the compartmentation of β-alanine in rat brain stem-spinal cord differed markedly, its Na^+-dependent binding compartment being equal to its total tissue content and its strychnine-sensitive binding accounting for about 0.06% of its total tissue content. Hence, greater proportions of tissue β-alanine than of GABA and glycine may be involved in its Na^+-dependent binding ('inactivation') and antagonist-sensitive binding ('receptor-interaction'). It is also noteworthy that all 3 amino acids had similar ratios of antagonist-sensitive/Na^+-dependent binding in the CNS regions studied.

These results strengthen the notion that the possible 'receptor-binding' of these inhibitory amino acids can be studied in the presence of physiological concentrations of Na^+. (Taurine has not yet been studied in detail). The value for BMI-sensitive GABA binding (60 pmoles/g cerebral cortex) agrees well with values obtained for the Na^+-independent binding of GABA to CNS membrane fractions[11,15,16]. The value for strychnine binding sites of 39 pmoles/g rat spinal cord[17] is lower than that determined for strychnine-sensitive glycine binding sites (160 pmoles/g spinal cord[4]), as was expected, since strychnine and glycine probably bind to distinct CNS sites[2,4,17]. 'High-affinity', strychnine-sensitive binding of β-alanine to a rat brain stem-spinal cord

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].

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

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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).