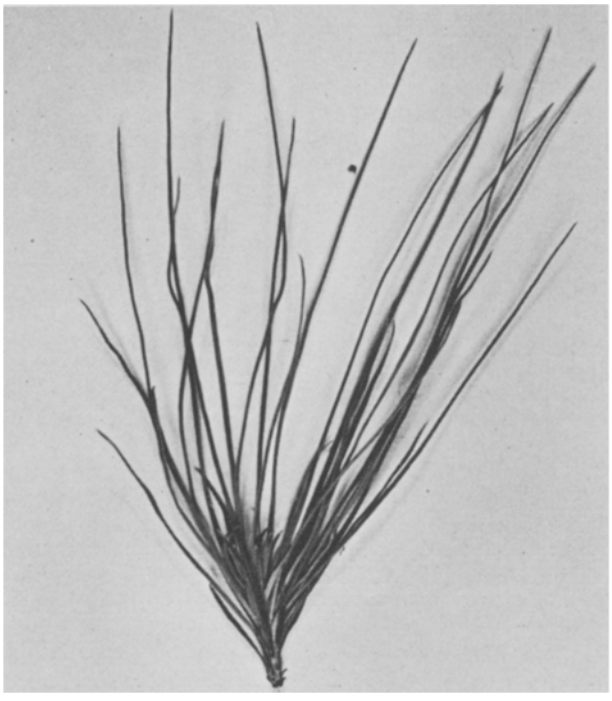

Fig. 1. A number of foliar leaves produced on a young long shoot of *Pinus roxburghii* (actual size).

Fig. 2. A number of foliar leaves and a dwarf shoot produced on an old long shoot of *Pinus roxburghii* (1/3 actual size).

seedlings[2-5]. It is reported[5] that as a *Pinus* seedling continues to develop, the later-formed foliar leaves on the main axis become progressively smaller and pass over gradually into the scale leaves of the mature stem. With our finding of the occurrence of green, needle-like, spirally-arranged, elongated, foliar leaves on long shoots of the adult plant, this *Pinus roxburghii* tree resembles the majority of gymnosperms.

These foliar leaves start developing on long shoots in early winter. They are elongated, measuring up to 10 cm, and are dull green with a bluish tinge in contrast to the needle leaves which are shining green. These leaves on long shoots have a slightly flattened base which gradually taper to the apex; sometimes, they are spirally twisted in bunches. Dwarf shoots arise in the axils of these green leaves. With the onset of hot weather in the month of April, when temperature shoots up to about 40 °C, they start drying, turn brown and then fall from the trees, leaving persistent leaf bases on the long shoots. Male cones develop quite profusely on this tree, but female cones have not been observed.

It appears that the general climate of the locality, which is very hot, is not congenial for the normal growth of the tree. Throughout the year, the temperature at Bundi is much higher than that of the usual habitat of this plant. During summer, it may reach up to 47 °C. The hot climate may be responsible for this abnormality. Possibly, this represents the reappearance of a character which is said to have been lost with the evolution of this plant[4].

1 Grateful thanks to Dr Elbert L. Little of the US Department of Agriculture (Forest Service) for suggestions.

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High-affinity binding of β-alanine to cerebral synaptosomes might involve glycine-receptors[1]

F.V. DeFeudis[2], L.M. Orensanz Muñoz, M.A. Vidal, G. Corrochano and M. Sanchez del Alamo

Centre de Neurochimie, 11 rue Humann, F-67085 Strasbourg Cédex (France), and Facultad de Medicina Universidad Autonoma and Centro 'Ramón y Cajal', Madrid (Spain), 14 February 1978

Summary. High-affinity, Na^+-independent binding of β-alanine to a synaptosomal fraction of rat brain was potently inhibited by glycine and by some other α-amino acids, but not by taurine or GABA. This binding mechanism, which was also sensitive to both bicuculline and strychnine, might involve synaptic receptors for both β-alanine and glycine.

β-Alanine, like GABA, glycine and taurine, meets most established criteria for the identification of mammalian CNS inhibitory neurotransmitters[3,4]. Its potent neuronal depressant action in vivo is antagonized by both strychnine and bicuculline in the thalamus and cerebral, hippocampal and cerebellar cortices[5,6]. β-Alanine is bound to synaptosome-enriched fractions of mammalian CNS tissues by Na^+-dependent, 'high-affinity' ($K_B \cong 5 \times 10^{-5}$ M) mechanisms[7,8] and by Na^+-independent, 'high-affinity' ($K_B \cong 5 \times 10^{-8}$ M), strychnine-sensitive mechanisms which could

represent its interaction with synaptic receptors[9]. In the present study, the substrate-specificity of high-affinity β-alanine binding has been examined in a cerebral synaptosome-enriched fraction in the absence of added Na^+.

Materials and methods. Male, Wistar rats (175–350 g) were decapitated, and their brains (rostral to the inferior colliculi; excluding cerebellum) were excised for preparation of a synaptosome-enriched fraction[10]. All operations with these P_2 fractions were conducted at 0 °C. First, they were resuspended in 3.0 ml of glucose-free, bicarbonate-buffered medium[11], which was made Na^+-free by substituting sucrose for 123 m-equiv/l of its contents of Na^+ and Cl^- and by substituting $KHCO_3$ for its $NaHCO_3$ content; osmolarity was 308 mosmoles/l and pH was 7.4 after equilibration with 95% O_2/5% Co_2. Then, aliquots (0.25 ml) of re-suspended fractions were pipetted into tared centrifuge tubes, and 0.2 ml of medium, either free of added substance or containing enough test substance to provide 10^{-3} M final concentrations, was added. After agitating the samples and allowing them to stand for 10–15 min, 0.5-ml aliquots of media, providing 5.4×10^{-9} M of ^{3}H-β-alanine (3-^{3}H-β-alanine; Radiochemical Centre, Amersham, Great Britain; 49 Ci/mmole) and 1.7×10^{-7} M of ^{14}C-sucrose (U-^{14}C-sucrose, Radiochemical Centre, Amersham; 610 mCi/mmole), were added. Samples were agitated, allowed to stand for 15 min, and centrifuged at 17,000 × g for 30 min to obtain final pellet and supernatant fractions. Radioactivity due to ^{3}H and ^{14}C and protein were determined as previously described[12,13]. ^{14}C-Sucrose distribution ratios were used to correct pellets for their contents of ^{3}H-β-alanine present as trapped supernatant fluid[10]. 'Non-specific' binding of β-alanine (i.e., radioactivity which remained in pellets in the presence of 10^{-3} M unlabelled β-alanine) was subtracted from all values to provide estimates of 'specific' binding[14]. Unlabelled substances were purchased from Calbiochem Corp. or Sigma Chemical Co., except for bicuculline-methiodide which was from Pierce Chemical Co.

Results and discussion. Pellet fractions weighed 12.1±1.2 (162) mg and contained 84.7±9.3 (159) mg protein/g; ^{14}C-sucrose distribution ratios were 0.63±0.01 (158); means±SD, numbers of samples in parentheses. Unlabelled β-alanine (10^{-3} M) reduced the amount of ^{3}H-β-alanine bound to the pellets by about 82%, thus providing an estimate of specific binding (table). It is noteworthy that α-amino acids (glycine, α-alanine, valine, leucine and α-aminobutyric acid) and β-aminoisobutyric acid were as potent, or nearly as potent, as α-alanine itself, at competing for ^{3}H-β-alanine binding sites. Hence, the receptor(s) involved appears to require a 1 or 2 carbon atom separation between carboxylic acid and amino functions. Omega-amino acids with functional group separations greater than 2 carbon atoms (e.g., GABA) did not compete with β-alanine for its receptor(s).

Modification of the carboxylic acid moiety of β-alanine (as with carnosine) and addition of acidic (as with L-glutamate) or basic (as with L-lysine) groups to the α-amino acid configuration also prevented interaction with β-alanine receptors. Taurine, the sulphonic acid analog of β-alanine, was also inactive. Hence, strychnine-sensitive taurine action in the vertebrate CNS may not be mediated by the receptors involved in strychnine-sensitive β-alanine action. In a previous report[15], taurine was also shown to be ineffective at competing for high-affinity glycine binding sites in a rat CNS preparation in the presence of a physiological concentration of Na^+. Both strychnine-SO_4 and bicuculline-methiodide potently inhibited β-alanine binding, in agreement with their significant blocking actions on β-alanine-induced neuronal depressions in higher CNS regions[5,6].

Since glycine and several other α-amino acids were as potent as β-alanine at competing for ^{3}H-β-alanine binding sites, it seems likely that glycine-receptors are involved in this binding. The finding that some α-amino acids which do not exert neuronal depressant actions (e.g., valine, leucine) were potent inhibitors of β-alanine binding may be useful to investigators who are designing iontophoretic studies aimed at localizing central glycine- and β-alanine-receptors.

Substrate-specificity of 'high-affinity' β-alanine binding; effects of some drugs

Substance (10^{-3} M)	^{3}H-β-Alanine bound (pmoles/g P_2)		Decrease in specific binding (%)
	Total	Specific	
None	6.24±0.31 (18)	5.13	–
β-Alanine	1.11±0.10 (9)*	–	100
Glycine	1.00±0.17 (3)*	0	100
DL-α-Alanine	1.25±0.51 (3)*	0.14	97
DL-β-Aminoisobutyric acid	1.11±0.22 (3)*	0	100
DL-α-Aminobutyric acid	1.30±0.18 (3)*	0.19	96
DL-Valine	1.43±0.02 (3)*	0.32	94
L-Leucine	2.42±0.20 (3)*	1.31	75
Taurine	5.29±0.56 (6)	4.18	19
γ-Aminobutyric acid	5.62±1.14 (3)	4.51	12
L-Carnosine	6.50±0.33 (3)	5.39	0
L-Lysine	7.70±0.46 (3)	6.59	0
L-Glutamate	7.48±0.51 (3)	6.37	0
Strychnine-SO_4	2.99±0.25 (3)*	1.88	63
Bicuculline-methiodide	3.49±0.24 (3)*	2.38	54

^{3}H-β-Alanine was present at 5.4×10^{-9} M; all pellets were corrected for trapped supernatant fluid using ^{14}C-sucrose distribution ratios[10]. Specific binding was determined by subtracting non-specific binding that occurred in the presence of 10^{-3} M unlabelled β-alanine. Means±SEM; * indicates $p<0.001$ between these values and controls (Student's t-test; two-tailed).

Note that the values given for β-alanine binding do not represent saturation binding (maximal specific binding), but were determined at 1 concentration only, namely 5.4×10^{-9} M.

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2 Visiting Professor and EMBO Fellow, Centre de Neurochimie, 11 rue Humann, F-67085 Strasbourg-Cédex, France.

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