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[^{3}H]Taurine binding on a cardiac sarcoplasmic fraction from rats, rabbits and pigs

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A subcellular fraction was isolated from heart ventricles by differential centrifugation. Enzyme markers and electron microscopy showed that this fraction was rich in sarcoplasmic reticulum. It bound [^{3}H]taurine specifically. Taurine might contribute to the regulation of Ca^{2+} transfer in the sarcoplasmic reticulum.

Taurine abounds in the mammalian heart. Its concentration is normally constant. Higher concentrations have been observed in animals and man and only in cardiac pathophysiological states such as hypertension with hypertrophy or congestive failure (Baskin & Finney 1979).

Taurine influx increases after stimulation of β-adrenoceptors (Huxtable et al 1980). Taurine has proved beneficial in treating congestive heart failure (Azuma et al 1983) and has an inotropic effect which depends on the extracellular calcium concentration (Huxtable & Sebring 1983). Moreover, it delays calcium efflux and increases uptake rate, as shown with calcium-free washout experiments (Huxtable & Sebring 1983). Taurine may affect calcium binding on sarcolemma, sarcoplasmic reticulum or mitochondria.

The effect of taurine on sarcolemmal calcium binding has already been studied by Chovan et al (1980) and by Read et al (1980). Two sets of taurine binding sites, high and low affinity, have been identified in rat heart sarcolemma (Kulakowski et al 1978). The interactions of the amino-acid with the low affinity site might modulate calcium binding on sarcolemma (Chovan et al 1980).

However, Franconi et al (1982) and Diacono & Dietrich (1976) have also proposed an intracellular action for taurine. Mitochondrial calcium binding might be affected by taurine (Huxtable & Sebring 1983). For the sarcoplasmic reticulum, results on the taurine calcium relation are still controversial (Chubb & Huxtable 1978; Entman et al 1977). We have investigated the ability of taurine to bind to sarcoplasmic structures.

Materials and methods

Compounds

KCl; $MgCl_2$ $6H_2O$; NaCl; KH_2PO_4; Na_2HPO_4 $12H_2O$; NaH_2PO_4 H_2O; $K_3Fe(CN)_6$; $Na_2S_2O_5$; Na_2SO_3; oxalic acid; ammoniumheptamolybdate; Tris; ethylenediaminetetraacetic acid (EDTA) (Merck). KCN; sodium succinate; H_2SO_4 (Prolabo). Albumin bovine, fraction V (Sigma). L-Histidine; taurine (Fluka). β-Nicotinamide-adenine dinucleotide phosphate reduced (NADPH); cytochrome c; glucose-6-phosphate disodium salt (Boehringer Mannheim). [^{3}H]Taurine (sp. act. 12·8 Ci mmol^{-1}, radiochemical purity 97%) (Amersham International).

Subcellular fraction preparation

Subcellular fractions were prepared according to a slightly modified Velema & Zaagsma procedure (1981). The hearts of Wistar rats or rabbits were quickly removed and immersed in 0·9% NaCl (saline). Pigs hearts obtained from slaughter were kept on ice. Cardiac ventricles were minced, washed in saline and homogenized in 8 volumes (rat and rabbit) or 3 (pig) of buffer consisting of 20 mM Tris HCl, pH = 7·1, 1 mM EDTA. All subsequent procedures were at 0 °C. An Ultra-turrax was used for homogenization at rheostat settings of 120 (rat or rabbit) or 150 (pig) for 4 periods of 5 s (rat), 3 periods of 7 s (rabbit) or 4 periods of 7 s (pig). Homogenization was completed in a Potter Elvehjem tissue grinder with 5 low (rat, rabbit) or medium (pig) speed treatments with motor-driven Teflon pestle.

The homogenate was twice strained through three layers of cheesecloth and then centrifuged at 8800*g* for 20 min to give a pellet P1. The resulting supernatant was recentrifuged at 12 500*g* for 20 min giving another pellet P2, and, similarly, supernatant 2 gave a pellet P3. Supernatant 3 was recentrifuged at 44 000*g* for 1 h. Pellet P4 was resuspended by hand homogenization with the Potter in 2 volumes (rat, rabbit) or 3/4 (pig) of 20 mM Tris-oxalate buffer solution, pH 6·8, 0·6 M KCl and 1 mM EDTA. The suspension, stirred for 5–10 min, was recentrifuged at 44 000*g* for 1 h. The resulting pellet P'4 was resuspended in 20 mM Tris-HCl buffer solution, pH = 7·0, 1 mM EDTA to obtain protein concentrations in the 1–2 mg ml^{-1} range. Protein concentration was measured according to Lowry et al (1951).

The fractions obtained were used either fresh ((Na^+, K^+)ATPase assay, glucose-6-phosphate assay, NADPH-cytochrome c reductase assay, taurine binding), diluted with glycerol and kept below −20 °C ((Na^+,K^+)ATPase assay, cytochrome c reductase assay, succinyl dehydrogenase assay) or kept in liquid nitrogen (succinyl dehydrogenase assay).

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Enzyme assays

(Na^+, K^+)ATPase activity. Na^+, K^+-ATPase activity was used as a sarcolemmal marker. The Na^+, K^+-ATPase activity was determined from the release of inorganic phosphate produced by ATP hydrolysis. The experimental conditions for this assay were as described by Schwartz et al (1973).

Inorganic phosphate was measured with the method of King (1938). Results were expressed as μmol of inorganic phosphate (Pi) released (mg protein)$^{-1}$ h^{-1}.

Glucose-6-phosphatase activity. Glucose-6-phosphatase was used as a marker for the sarcoplasmic reticulum and its activity was assessed from the rate of inorganic phosphate release from glucose 6-phosphate. This assay was performed according to Aronson & Touster (1974). Inorganic phosphate was determined according to King (1938). Results were expressed as μmol Pi (mg protein)$^{-1}$ h^{-1}.

NADPH-cytochrome c reductase activity. This enzyme has frequently served as an alternative marker to glucose 6-phosphatase activity. Enzyme activity was determined with the method of Sottocasa et al (1967). Cytochrome c reduction was monitored spectrophotometrically at 550 nm. Results were expressed as μmol reduced cytochrome c (mg protein)$^{-1}$ h^{-1}.

Succinyl dehydrogenase. Succinyl dehydrogenase was used as a mitochondrial marker. Enzyme activity was determined with a spectrophotometric method, using $K_3Fe(CN)_6$ as an electron acceptor.

Assays were performed with a slightly modified version of the method described by Veeger et al (1969): the $K_3Fe(CN)_6$ concentration was halved and the reaction was followed at 420 nm, instead of 455 nm, for 15 min.

Results were expressed as μmol of fumarate (mg protein)$^{-1}$ h^{-1}.

[^{3}H]Taurine binding

Binding assays were performed by incubating in siliconized tubes, 100 μl of 20 mM Tris-HCl buffer solution, pH = 7·7, 236 mM NaCl, 250 nM [^{3}H]taurine with 100 μl subcellular preparation under shaking for 75 min at 20 °C. Non-specific binding was determined by addition of 2·5 mM unlabelled taurine.

The assays were terminated by rapid filtration under reduced pressure on Whatman GF/B filters. The filters were rinsed with 15 ml 20 mM Tris-HCl, 118 mM NaCl buffer solution, pH = 7·5, at 20 °C. They were then transferred to vials containing 16 ml of Biofluor (New England Nuclear) and counted by liquid scintillation spectrometry.

Results

Radioactivity due to [^{3}H]taurine was found essentially in the P'4 pellet. [^{3}H]Taurine specifically bound amounted to 30 to 50% of total binding according to the species studied (Table 1).

Taurine binding in the P'4 fraction was up to 14 times higher than in the homogenate, for the rat, and similar differences were observed for the other species (Table 2).

Enzyme studies are summarized in Table 2. Enhanced activity of the sarcoplasmic reticulum markers is observed in P'4, but this increase, found in all the species studied, was never more than 7 times that found for the homogenate. Succinyl dehydrogenase was always slightly lower. (Na^+, K^+)ATPase activity never increased. P'4 pellet was also characterized as sarcoplasmic reticulum under the electron microscope. As seen in Fig. 1, this sarcoplasmic reticulum appeared as a homogeneous vesicular preparation.

Discussion

Our subcellular preparation gives a sarcoplasmic fraction binding of [^{3}H]taurine 14 times as much as the original homogenate, at least for the heart preparation.

A saturation test was made with a rat heart preparation, but the [^{3}H]taurine concentrations used (100–1000 nM) were not sufficient for saturation or for valid analysis with a Scatchard plot. It was impossible to determine whether it was a single receptor population or not. However, assuming one set of sites in the range

Table 1. [^{3}H]Taurine specifically bound to different fractions from rat, pig and rabbit heart.

	Rat (n = 6)		Pig (n = 3)		Rabbit (n = 3)	
	Specific binding fmol (mg prot.)$^{-1}$	% SP	Specific binding fmol (mg prot.)$^{-1}$	% SP	Specific binding fmol (mg prot.)$^{-1}$	% SP
Homogenate	41 ± 11	9 ± 2	30 ± 15	3 ± 1	44 ± 16	9 ± 3
P1	13 ± 2	5 ± 1	79 ± 49	8 ± 4	15 ± 8	3 ± 2
P2	131 ± 13	17 ± 3	85 ± 9	6 ± 1	179 ± 56	25 ± 7
P3	76 ± 18	10 ± 4	89 ± 41	6 ± 2	Non-measurable	Non-measurable
P'4	597 ± 35	38 ± 3	187 ± 9	30 ± 1	359 ± 10	52 ± 1
P5	48 ± 12	6 ± 3	7 ± 3	1 ± 0·5	Non-measurable	Non-measurable

n = Assay number. P5 = Recentrifuged supernatant 4 at 77 000*g* for 1 h.

Table 2. [^{3}H]Taurine binding and enzyme assays of homogenate and P'4 fraction prepared from rat, rabbit and pig heart.

		[^{3}H]Taurine binding fmol (mg prot.)$^{-1}$	NADPH-cyt c reductase µmol cyt c reductase (mg prot.)$^{-1}$ h^{-1}	Glucose-6-phosphatase µmol Pi (mg prot.)$^{-1}$ h^{-1}	Succinyl dehydrogenase µmol fumarate (mg prot.)$^{-1}$ h^{-1}	(Na^+, K^+) ATPase µmol Pi (mg prot.)$^{-1}$ h^{-1}
Rat	Homogenate	n = 9 41 ± 11	n = 4 0·24 ± 0·01	n = 7 0·11 ± 0·01	n = 3 9·1 ± 0·3	n = 7 1·1 ± 0·4
	P'4	n = 9 567 ± 27	n = 4 1·04 ± 0·05	n = 7 0·92 ± 0·22	n = 2 6·16	n = 7 0·1 ± 0·1
Rabbit	Homogenate	n = 6 43 ± 18	n = 9 0·20 ± 0·02	n = 2 0·16	n = 7 9·5 ± 1·1	n = 4 1·6 ± 0·4
	P'4	n = 6 383 ± 21	n = 5 0·34 ± 0·04	n = 2 0·75	n = 3 5·6 ± 1·1	n = 4 1·0 ± 0·8
Pig	Homogenate	n = 12 19 ± 5	n = 6 0·20 ± 0·02	—	n = 5 34·8 ± 3·6	n = 4 2·5 ± 1·0
	P'4	n = 12 264 ± 20	n = 7 1·43 ± 0·12	—	n = 2 5·09	n = 4 2·2 ± 1·3

n = Assay number.

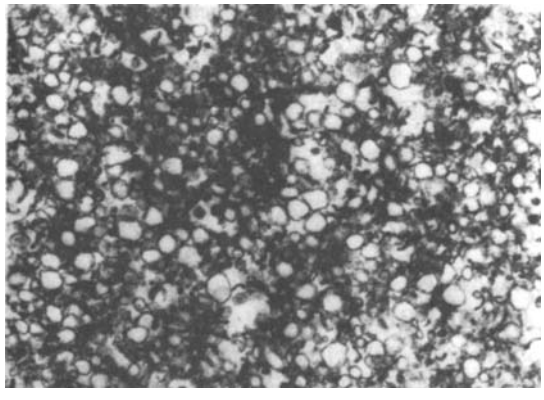

FIG. 1. Sarcoplasmic reticulum fraction observed under the electron microscope.

of concentrations studied, [^{3}H]taurine binding was characterized by a K_D of 3·2 µM and a B_{max} of 7·25 pmol (mg protein)$^{-1}$. These results tally with those of Franconi et al (1981) who, for sarcolemma from guinea-pig heart, report a K_D of 1·94 µM and a B_{max} of 3·05 pmol (mg protein)$^{-1}$ (values obtained for the high affinity sites). López-Colomé & Pasantes-Morales (1981) found similar binding characteristics for rat brain cortex (K_D = 4·06 µM and B_{max} = 5·36 pmol (mg protein)$^{-1}$).

On the other hand, Kulakowski et al (1978) described a high affinity site with binding characteristics different from those of other authors.

Our results support the hypothesis of intracellular taurine receptors with characteristics similar to those of membrane receptors.

The observation that the sarcoplasmic reticulum binds taurine is of great interest and tallies with the theory of Dolara et al (1978) that an adequate cardiac Ca^{2+} level is partially regulated by the sarcoplasmic reticulum via taurine. They suggested that this amino-acid might modify calcium turnover by the formation of a ternary system between taurine, Ca^{2+} and cellular macromolecules, among which might be calsequestrin. However, it is possible that taurine may act directly by modifying the sarcoplasmic reticulum membrane conformation, as has been proposed for sarcolemma, since there are structural similarities between this amino-acid and the zwitterionic group of phospholipids. It is also possible that taurine may act as a calmodulin inhibitor as it was found to do in modulating myocardial metabolism (Schaffer et al 1983). Moreover, cGMP is known to influence the movement of Ca^{2+} in the sarcoplasmic reticulum and, under certain conditions, guanylate cyclase is activated by taurine (Mal'Chikova & Elizarova 1981).

Conclusion

Electron microscopy on one hand, and (Na^+, K^+)ATP-ase, succinyl dehydrogenase, cytochrome c reductase, glucose-6-phosphatase used as enzyme markers on the other, characterized the fraction isolated at 44 000*g* as sarcoplasmic reticulum.

This fraction bound 14 times more [^{3}H]taurine than the original homogenate.

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Letters to the Editor

Proposal regarding opioid anomalies

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Opioid activity associated with the (+)-isomer of a structurally rigid molecule such as a 3-benzazocine (6,7-benzomorphan) is considered abnormal. However, extensive studies of the optical isomers of 1,2,3,4,5,6-hexahydro-3,6,11-trimethyl-2,6-methano-3-benzazocine-8-ol (metazocine) and certain of its homologues, revealed a remarkable profile of activity (Fraser et al 1962; Ager et al 1969; Kosterlitz 1969; Villarreal 1970). It was found that the (−)-isomers had potent antinociceptive activity in the hot-plate assay and precipitated abstinence in morphine-dependent monkeys. On the other hand, four of the five corresponding (+)-isomers also had antinociceptive properties in mice. Indeed, although the (+)-isomers were always less potent than their respective enantiomers, the difference was as little as six-fold for the 6,11-diethyl compound. Interestingly, the (+)-isomers were all inactive as antagonists and all of the homologues with ethyl or propyl groups at carbon 6 substituted for morphine in addicted monkeys. Thus, these active (+)-isomers were clearly manifesting what has been designated as mu opioid properties.

Although a rather ingenious opioid receptor model which explained similarities in pharmacological properties of opioids with apparently dissimilar chemical structures was proposed (Feinberg et al 1976), and this model could help understand certain stereochemical paradoxes, the issue of antipodal (enantiomeric) anomalies was not addressed. In an attempt to explain certain stereochemical abnormalities, another worker modified this model (Galt 1977). The modification involved an extension of the planar binding site (A) rather than the presence of an additional lipophilic region (F) of the model.

Knowing that there is a spectrum of activity ranging from absolute stereospecificity for the optical isomers of morphine to anomalous activity for the optical isomers of metazocine and its homologues, and because we felt that some explanation must exist, we decided to build Dreiding models of the antipodes of metazocine and morphine, and of enantiomers of the morphinan series levorphanol and dextrorphan to determine firsthand what features they had in common or for that matter how they were dissimilar. Once the stereomodels of metazocine were built, we noted that the piperidine rings of (+)- and (−)-metazocine (Figs 1 and 2, respectively) could be superimposed (Fig. 3) and that as a result, the following carbon atoms were juxtapositioned; namely, (+)- and (−)-7, (+)- and (−)-8

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