Tritiated water (HTO) and inulin spaces in isolated skeletal and cardiac muscles: influence of contractile activity[1]

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Summary. In isolated cardiac and skeletal muscle inulin space increased significantly after isometric contractions: no significant change (in myocardium) or a less pronounced increase (in skeletal preparations) was found following isotonic responses. The HTO space was uninfluenced by the contractile activity.

Changes in the size of the extracellular space have been shown to take place following mechanical activity in vivo of skeletal muscle[2,3] and modifications of the contractile state of perfused myocardium[4].

Few data are available as to whether the observed changes also depend on the mechanical type of contraction (isotonic or isometric). In the present study the total muscle water content and the extent of extracellular space both in quiescent and active (isometric or isotonic contractions) skeletal and cardiac muscles were evaluated. The experiments were carried out on isolated preparations. However it must be noted that inferences about the in vivo state based on measurements of fluid compartments made on in vitro preparations should be made with considerable caution[4–6].

Materials and methods. Skeletal (soleus) and cardiac (papillary) muscle preparations were isolated from Wistar rats (60 g b.wt for the soleus, 200 g b.wt for the papillary muscle) under light ether anaesthesia. The muscles were mounted in a small (15 ml) thermoregulated (20 °C) bath filled with Krebs-Henseleit[7] solution, containing tritiated water (HTO) 0.5 μCi/ml (Radiochemical Centre - Amersham) or inulin (10 mg/ml) (RP, Erba, Milan) and bubbled with 5% CO_2 in O_2. One end of the specimens was tied to a force transducer (Statham G1, 1.5–300) and the other one to a light isotonic lever fitted with a linear photoelectric displacement transducer.

All the preparations were stretched to l_{max} (muscle length at which developed tension was maximum) and then maintained at rest for 90 min. Then, some muscles were stimulated (supramaximal stimuli) to contract in isometric or isotonic (only the preload was applied on the lever) conditions, according to the following schedule: a) soleus muscle: a set of 5 tetanic contractions (50 stim/sec; 1-min duration) at 1-min intervals; b) papillary muscle: 20-min stimulation (frequency 2/min). For both types of preparation, control muscles remained at rest for the same experimental periods (i.e., 9 min for the soleus and 20 min for the papillary muscle).

Muscle HTO uptake was measured as follows: radioactive bathing solution adhering to the preparation surface was removed by gently blotting twice with filter paper (Whatman No. 5). The muscle was then weighed on a torsion balance and placed in a vial containing 5 ml of non-radioactive Krebs-Henseleit solution ('wash-out' solution), where the specimen was maintained for 18 h and frequently stirred. A sample of the wash-out solution was transferred to a vial containing scintillation fluid (Instagel, Packard) for the radioactivity measurement, and counted with a liquid scintillation counter (Packard Tri-Carb, model 3385). Muscle inulin uptake was evaluated after removing inulin solution adhering to the preparation surface. This was done by dipping the muscle rapidly into 3 successive beakers containing Krebs-Henseleit solution without inulin and by blotting it twice with wet gauze. Inulin was determined according to the method of Ross and Mokotoff[8].

Results and discussion. From the table it can be observed that: a) In resting conditions a higher value for extracellular space (inulin space) was found in cardiac than in skeletal muscle, as reported by Poole-Wilson and Cameron[9]. b) Stimulation significantly increased inulin space in the soleus. The increase was more pronounced after isometric than isotonic contractions. These findings confirm the observations reported on in vivo skeletal muscle[3]. In papillary muscle an increase of inulin space was found after isometric contractions, while there was no significant change following isotonic responses. Poole-Wilson et al.[4] observed a reduction of extracellular space, as a consequence of activity of cardiac muscle; however the extracellular volume was estimated by a different method and utilizing, as a marker, ^{51}Cr-EDTA, with which larger distribution volumes are obtained than with inulin. c) The total water (HTO space) content of the tissues is similar to the water space evaluated by Law and Phelps[10] and is only slightly less than that obtained by determining muscle weight before and after drying[2,9]. d) In both preparations the HTO space was uninfluenced by the contractile activity.

This last finding suggests that the apparent increase of the extracellular space, observed following muscular activity (only isometric for cardiac muscle), might be caused by the

Inulin and HTO distribution spaces (ml/100 g w.wt) of the soleus and papillary muscles in different experimental conditions

Experimental conditions	Inulin space				HTO space			
	n	Soleus	n	Papillary	n	Soleus	n	Papillary
A Rest	28	13.44 ± 0.59	24	18.18 ± 1.16	20	71.20 ± 1.88	20	67.38 ± 1.78
B Contractions (isometric + isotonic)	56	17.12 ± 0.69	48	22.17 ± 1.04	40	68.91 ± 1.14	40	69.10 ± 1.56
C Isometric contractions	28	18.14 ± 1.13	24	24.82 ± 1.56	20	67.98 ± 1.39	20	69.57 ± 2.02
D Isotonic contractions	28	16.09 ± 0.77	24	19.51 ± 1.18	20	69.85 ± 1.81	20	68.63 ± 2.42

Inulin space:	Soleus	Papillary
B vs A	$p \leq 0.01$	$p \leq 0.01$
C vs A	$p \leq 0.01$	$p \leq 0.01$
D vs A	$p \leq 0.05$	NS
C vs D	$p \leq 0.05$	$p \leq 0.01$

Means ± SE; n, number of preparations. HTO space: no statistical differences were found between different experimental conditions. Statistical significance has been calculated according to Student's t-test.

shift of a certain amount of water from the intracellular to the extracellular space.

However the results obtained do not indicate whether the increase of inulin uptake due to contractile activity can be attributed to an actual change of volume of the extracellular space rather than to a modification in the diffusion kinetics of inulin: in this latter case we must assume that non equilibrium conditions are present[11]. During contractions, inulin might be taken up more easily by compartments (perhaps other than the extracellular space) where, in resting conditions, it enters at a very low rate: it seems difficult to explain why the phenomenon should be less pronounced or absent following isotonic contractions.

In order to clarify the results obtained, a further investigation will be devoted to the diffusion kinetics of inulin and HTO in resting and active muscles. Anyhow the results obtained suggest that the determination of cellular electrolytes in isolated muscle preparations should be accompanied by an accurate measurement of the extracellular space and its possible changes due to the experimental conditions.

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A new neurosecretory system in fish, located in the gill region

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Summary. A peculiar neurosecretory system is reported in 6 teleost species; *Clarias batrachus, Heteropneustes fossilis, Mystus seenghala, Ompak pabda, Glassogobius giuris* and *Notopterus notopterus.* It is located in the gill region close to the pseudobranch or to the carotid labyrinth. The neurosecretory cells have been identified using stains specific for neurosecretion. The results are discussed in the light of the association of the neurosecretory system with the pseudobranch or the carotid labyrinth, and the airbreathing habit of these fishes.

In fishes, only 2 neurosecretory systems are known; the hypophysial system of the head and the caudal neurosecretory system of the tail. A peculiar 3rd system of neurosecretion has been found by us to exist in certain fishes, and in the present report an account of this is presented. The neurosecretory cells have been identified histologically, using neurosecretory stains like aldehyde fuchsin[2], acid-violet[3] and iron-haematoxylin[4]. So far, this system has been found in 6 teleostean species; namely *Clarias batrachus, Heteropneustes fossilis, Mystus seenghala, Ompak pabda, Glassogobius giuris* and *Notopterus notopterus,* which belong to 3 different orders, but not in the carps *Labeo rohita* and *Cirrhinus mrigala.*

Results. In the 6 species mentioned above, neurosecretory cells are found to occur clumped into groups forming a large ganglionic mass, which is located in the gill region in

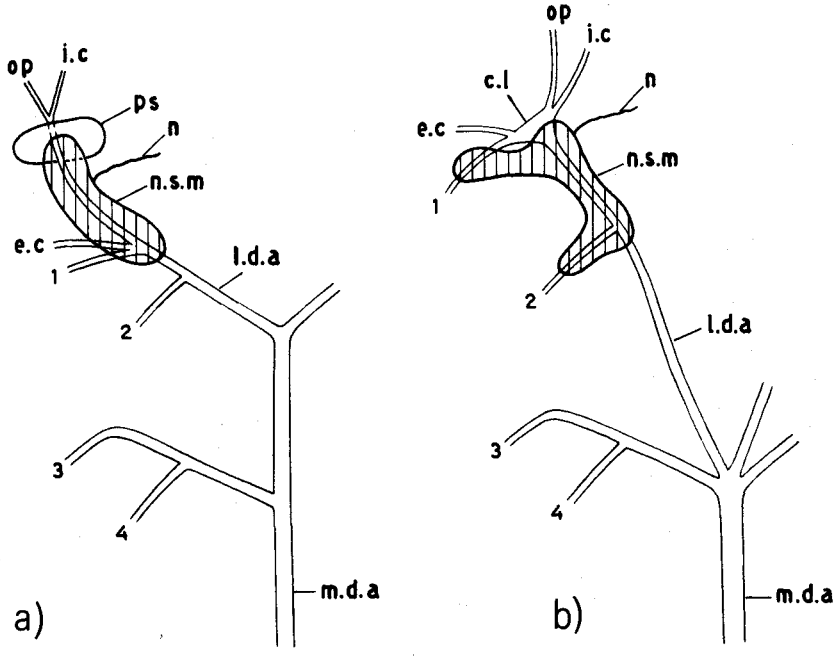

Figure 1. Schematic drawings (ventral view) showing the location of the pseudobranchial neurosecretory mass in *Glossogobius giuris* (a) and in *Clarias batrachus* (b).

Abbreviations used: 1, 1st efferent branchial artery; 2, 2nd efferent branchial artery; 3, 3rd efferent branchial artery; 4, 4th efferent branchial artery; c.l, carotid labyrinth; e.c, external carotid artery; i.c, internal carotid artery; l.d.a, lateral dorsal aorta; m.d.a, median dorsal aorta; n, branch of glossopharyngeal nerve; n.s.m, neurosecretory mass; op, ophthalmic artery; ps, pseudobranch.