Isolated rat seminiferous tubules synthesize guanidoacetic acid and creatine from arginine

NIGEL P. MOORE,* JOHN A. TIMBRELL* and TIM J. B. GRAY†

*Toxicology Unit, The School of Pharmacy, 29/39 Brunswick Square, London WC1N 1AX, U.K., and †British Industrial Biological Research Association, Woodmansterne Road, Carshalton, Surrey SM5 4DS, U.K.

Creatine is synthesized in a two-stage process, involving transfer of the amidine group of arginine to glycine to form guanidoacetic acid, which is subsequently methylated to creatine. The first report of testicular creatine synthesis was made by Alekseeva & Arkhangel’skaya (1964) based upon experiments in which whole testicular tissue was incubated in vitro with [1-14C]arginine. These findings were corroborated by studies *in vivo* in which [14C]arginine or [1-14C]guanidoacetate was injected directly into the testes of anaesthetized rats (Koszalka, 1968). However, in these studies no consideration was given to the localization of creatine synthesis within the organ.

Using cell-specific testicular toxins and monitoring urinary creatine levels, we have shown that testicular creatine in the rat is associated with the seminiferous epithelium, and with the germ cells in particular (Rawcliffe *et al.*, 1988; N. P. Moore, D. M. Creasy, T. J. B. Gray & J. A. Timbrell, unpublished work). This led us to consider that creatine metabolism may play a role in the normal function of the seminiferous epithelium.

The seminiferous tubules are highly metabolically active structures (e.g. Grootegoed *et al.*, 1988) and the purpose of this investigation was to determine whether they synthesize creatine, as a preliminary to the study of inter- and intracellular creatine metabolism.

The testes from 16-17-week-old Hsd/Ola: Sprague-Dawley rats (Olac) were decapsulated and the seminiferous tubules were separated from the interstitial tissue by collagenase digestion essentially as described by Jutter *et al.* (1982).

Tubules, or the unpurified interstitial cell suspension, were incubated for 3 h at 32°C in a shaking water bath and in a 5% CO2:95% O2 atmosphere. The incubation medium was Hank’s balanced salt solution, supplemented with glycine (0.15 mg/ml) and l-methionine (0.298 mg/ml), and containing 1-14C-arginine (0.1 μCi/ml, 54.4 mCi/mmol; Amersham U.K.). At the end of the incubation, the tissue was homogenized by sonication (MSE Soniprep 150), boiled for 5 min and centrifuged at 2000 g for 10 min. A 50 μl aliquot of the supernatant was applied to a C18 reverse-phase h.p.l.c. column (MCH10 ODS, Varian) fitted with a guard column, and maintained at ambient temperature. The mobile phase was an aqueous solution of Na2SO4 (10 mM), H2SO4 (5 mM) and sodium 1-hexanesulphonate (5 mM). Arginine and its metabolites were separated by a flow program [flow rate = 1 ml/min for 8 min, increased to 2 ml/min (duration 1 min) for 4 min, and increased to 4 ml/min (duration 2 min) for a further 16 min]. Fractions were collected every 0.4 min and the radioactivity was determined by liquid scintillation counting.

A typical h.p.l.c. profile, obtained from the incubation of isolated seminiferous tubules with guanido-radio labelled l-arginine as described above, is shown in Fig. 1. The major peak has a retention time of 11.6 min and is due to unmethylated arginine; the two earlier eluting peaks (I and II) have retention times of 6.3 and 7.2 min and co-chromatograph with standards of guanidoacetate and creatine, respectively.

Under the conditions described, seminiferous tubules extensively incorporated the amidine group of arginine into both guanidoacetate and creatine, accounting for 13.2 ± 3.8% and 18.5 ± 5.6% of the soluble radioactivity, respectively (values are mean ± s.d., n = 11, from three separate tissue preparations). The unpurified interstitial cell suspensions did not synthesize guanidoacetate or creatine to the same extent (1.3 ± 0.3% and 1.9 ± 0.5% of the soluble radioactivity respectively, mean ± s.d., n = 7, from two separate tissue preparations), and such incorporation is probably due to contaminating tubular cells. Tubular tissue that had been boiled for 5 min before incubation with 1-14C-arginine did not synthesize [14C]guanidoacetate nor [14C]creatine.

In summary, the data presented in this communication are in agreement with previously published observations on the ability of the mammalian testis to carry out both stages of creatine synthesis (*i.e.* transamidination between arginine and glycine to produce guanidoacetate, which is subsequently methylated to creatine), but in addition show that such synthesis is localized within the seminiferous tubular compartment. The preliminary work described here is now being further investigated in our laboratory, using purified cell cultures, in an effort to elucidate the cellular site(s) of creatine synthesis and utilization within the seminiferous epithelium.

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[\textsuperscript{32}P]P\textsubscript{i} uptake in cultured fibroblasts and osteoblasts: most of the cellular [\textsuperscript{32}P] is not P\textsubscript{i}

GRAHAM J. KEMP*, ALAN BEVINGTON,\dagger HAMED I. KHOUJA and R. GRAHAM G. RUSSELL

Department of Human Metabolism and Clinical Biochemistry, University of Sheffield Medical School, Beech Hill Road, Sheffield S10 2RX, U.K.

There is mounting evidence that changes in P\textsubscript{i} metabolism accompany and may mediate stimulation of mammalian cells by hormones and other factors (Medina & Illingworth, 1984). Such changes are commonly studied by labelling with [\textsuperscript{32}P]P\textsubscript{i}, but even in the simple case of the human erythrocyte (with no intracellular organelles), [\textsuperscript{32}P] labelling kinetics are complicated by the presence of more than one cellular P\textsubscript{i} pool (Kemp et al., 1986, 1987a, 1988a). Therefore to interpret properly the hormonal dependence of [\textsuperscript{32}P]P\textsubscript{i} labelling in multicompartmental cells, studies are needed (like these in the erythrocyte) of apparent compartmentation of P\textsubscript{i} metabolism. We report here [\textsuperscript{32}P]P\textsubscript{i}-labelling studies of two cultured cell lines: the osteoblast model osteosarcoma UMR 106-01 and the human fibroblast line Detroit 532 (ATCC no. 54).

[\textsuperscript{32}P]P\textsubscript{i} was added to cells incubated on 12-well culture plates in growth medium or a Tris-buffered Ringer solution, and at time \( t \) cells were rapidly washed and deproteinized on the culture plate, as described elsewhere (Bevington et al., 1987a). Neutralized perchloric acid cell extracts were assayed for P\textsubscript{i}, [\textsuperscript{32}P]P\textsubscript{i} and [\textsuperscript{32}P] in organic phosphates (OP) as described elsewhere (Kemp et al., 1986).

In experiments to study the kinetics of [\textsuperscript{32}P] P\textsubscript{i} uptake, in UMR 106-01 incubated for up to 3 h in growth medium (\( n = 7 \)) the specific activity of cellular P\textsubscript{i} (\( a_1 \)) at time \( t \), relative to that of extracellular P\textsubscript{i} (\( a_0 \)), rose to a stable plateau of \( 0.27 \pm 0.02 \) (mean \( \pm \) s.e.m.) with initial rate \( 0.74 \pm 0.06 \) h\(^{-1} \) and half-time 14 \( \pm \) 1 min. This is consistent with a simple model of phosphate metabolism (Kemp et al., 1988b), in which cellular P\textsubscript{i} (pool 2) exchanges with extracellular P\textsubscript{i} (pool 1) at rate \( J = k \left[ P\textsubscript{i}\right] \), where \( k \) is the flux constant and \( \left[ P\textsubscript{i}\right] \) is the total cellular [\textsuperscript{32}P]P\textsubscript{i} concentration (0.3-3.7 mM). The Na\textsuperscript+-dependent components of both fluxes were saturable, and both had \( k_m = 0.8 \pm 0.2 \) mmol/litre of cell water (Na\textsuperscript{-}-dependent) and \( 0.65 \pm 0.04 \) mmol/litre of cell water (Na\textsuperscript{-}-independent). In the Na\textsuperscript{-}-dependent component, the flux \( J \) into OP was 1.1 \pm 0.3 mmol/h per litre of cell water (Na\textsuperscript{-}-independent). The decay rate of the Na\textsuperscript{-}-dependent fraction of P\textsubscript{i} influx is into cellular P\textsubscript{i}, rather than OP. This is the case, but the fraction of Na\textsuperscript{-}-dependent influx into cellular P\textsubscript{i} is surprisingly small: in UMR 106-01 at 1 nM-extracellular Na\textsuperscript{+}, flux \( I \) into cell P\textsubscript{i} was \( 1.9 \pm 0.1 \) mmol/h per litre of cell water (Na\textsuperscript{-}-dependent) and \( 0.65 \pm 0.04 \) mmol/h per litre of cell water (Na\textsuperscript{-}-independent).

Finally, it has been suggested that, in erythrocytes (Shoemaker et al., 1988) labelling of a membrane-associated OP pool occurs predominantly through a Na\textsuperscript{-} linked P transport system, while labelling of cellular P\textsubscript{i} occurs mainly through a passive Na\textsuperscript{-}-independent transporter. We therefore studied the Na\textsuperscript{-}-dependence of cellular P\textsubscript{i} and OP labelling fluxes in UMR 106-01 \(( n = 12)\) over a range of extracellular P\textsubscript{i} concentrations (0.3-3.7 mM). The Na\textsuperscript{-}-dependent components of both fluxes were saturable, and both had \( k_m = 0.8 \pm 0.2 \) mmol/litre of cell water, and \( V_{\text{max}} = 2.4 \pm 0.5 \) times the flux at 1 mm-P\textsubscript{i}. The Na\textsuperscript{-}-independent components of both fluxes increased linearly with extracellular P\textsubscript{i}. As a result the Na\textsuperscript{-}-independent fractions of both fluxes increased with extracellular P\textsubscript{i}; for \( I \) this fraction was 23 \pm 3% at 0.3 mm-P\textsubscript{i}, 26 \pm 5% at 1 mm and 50 \pm 7% at 3.7 mm; for \( J \) it was 6 \pm 1%, 8 \pm 1% and 19 \pm 7%.

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\*Present address: M.R.C. Biochemical and Clinical Magnetic Resonance Unit, John Radcliffe Hospital, Oxford OX3 9DU, U.K.

\daggerTo whom correspondence should be addressed.

Abbreviations used: OP, organic phosphate; \( a_1 \), activity of [\textsuperscript{32}P]P\textsubscript{i} (c.p.m./l) in phosphorus pool; \( a_c \), the corresponding [\textsuperscript{32}P] specific activity (c.p.m./mmol); \( I \) and \( J \), P\textsubscript{i} fluxes (mmol/h per litre of cell water).

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