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SPATIAL LOCALISATION OF MAGNETIC RESONANCE SPECTRA FROM NEONATAL BRAINS. Peter L. Hope, Martin J. Blackledge, N. Kevin Ives, Bheeshma Rajagopalan, Philip Sutton, George K. Radda. University of Oxford, John Radcliffe Hospital, Dept of Paediatrics and MRC Clinical MR Facility, Oxford, England

Studies of cerebral metabolism using phosphorus magnetic resonance spectroscopy (^{31}P MRS) can be refined using phase modulated rotating frame imaging (PMRFI) for spatial localisation. Six full term infants were studied at 1-9 days of age, in a 1.9 tesla magnet, using a 6.5cm diameter radiofrequency receiver coil overlying the temporo-parietal skull. Conventional MRS was used to obtain metabolite ratios from a large volume of one hemisphere. Spectra were then obtained, using PMRFI, from 6.5cm diameter slices of cerebral tissue at increasing depths up to 3.5cm into the brain with 0.6cm depth resolution.

Global phosphocreatine/inorganic phosphate (PCr/Pi) ratios from 4 mildly asphyxiated infants were between 1.54 and 4.35. Two babies with severe asphyxia had ratios of 0.55 and 0.86. The PMRF images from these two infants showed considerable heterogeneity, the PCr/Pi ratios from slices 2cm deep to the coil being 44% and 43% of the ratios from more superficial slices. The region of maximally impaired metabolism corresponds to the subcortical white matter known to be vulnerable to ischaemia.

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BILIRUBIN NEUROTOXICITY AND BLOOD-BRAIN BARRIER OPENING IN THE RAT STUDIED USING ^{31}P MAGNETIC RESONANCE SPECTROSCOPY (^{31}P MRS). N. Kevin Ives, Nicholas M. Bolas, R. Mark Gardiner. (Spon. by Peter L. Hope). University of Oxford, John Radcliffe Hospital, Dept of Paediatrics and MRC Clinical MR Facility, Oxford

^{31}P MRS was used to investigate disruption of brain energy status by a combination of hyperbilirubinaemia and an open blood-brain barrier (BBB). Spectra were acquired using a surface coil over the right cerebral hemisphere of anaesthetised adult male rats. Observations were made before and after intravenous infusion of bilirubin (BR, 5.2mM, 10ml in 400μM bovine serum albumin) or a control albumin solution, and after hyperosmolar BBB opening with l-arabinose (1.8 Molal) infused via the right external carotid artery over 20s at 7.5ml/min. Results are expressed as the ratio of phosphocreatine (PCr) to PCr + inorganic phosphate (Mean ± SD).

	Baseline	BBB closed	BBB open	
Control (n=8)	0.74±0.03	0.76±0.04	0.63±0.12	P < 0.001 Control vs BR (ANOVA)
BR (n=9)	0.70±0.05	0.68±0.06	0.44±0.14	

These observations provide evidence in vivo that bilirubin affects cerebral energy metabolism in the presence of an open BBB.

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CONCENTRATION-DEPENDENT INHIBITORY EFFECT OF BILIRUBIN (B) ON PHOSPHORYLATION OF SOME PROTEINS. T.W.R.Hansen, S.I.Walaas. Dept. of Ped. Res., U of Oslo, and Lab. for Molec. and Cell. Neurosci., The Rockefeller U, New York,N.Y. Protein phosphorylation appears to play a central role in regulation of cell processes. We have studied the effects of B 1-320μM on phosphorylation of some purified proteins by different kinases. The proteins in concentrations of 100μg/ml were phosphorylated at 30°C by 10nM concentrations of the kinases with 5μM ATP-^{32}P (148GBq/mM) as phosphate donor. The reactions were stopped after 30s, and phosphorylation was quantitated by scintillation counting of gel pieces after SDS-polyacrylamide gel electrophoresis. B inhibited phosphorylation of the following proteins: Synapsin I by cAMP-dependent kinase (APK) and Ca/calmodulin-dependent kinase II (CaMII); glycogen synthetase by APK and CaMII; histone I by C-kinase; histone IIA by APK, CaMII,and G-kinase (GK); DARPP-32 by APK and GK; and myosin by CaMII; but not G-substrate by GK. IC_{50} concentrations of B varied from 20-240μM. B may be capable of interfering with a wide range of protein phosphorylation reactions involved in regulation of cell metabolism.

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PREDICTION OF PERINATAL BRAIN DAMAGE BY CORD PLASMA VASOPRESSIN (AVP), ERYTHROPOIETIN (EP), AND HYPOXANTHINE (HX) Vineta Ruth, Kari O. Raivio, Children's Hospital, University of Helsinki, Helsinki, Finland. The classical criteria of asphyxia, Apgar score and metabolic acidosis are poor predictors of perinatal brain damage. Our aim was to assess if AVP (released after stress or asphyxia), EP (synthesized after hypoxic stimulus), and HX (an ATP degradation product) are better predictors.

We measured AVP and HX in umbilical arterial (UA) and EP in venous plasma of 62 infants born after preeclampsia pregnancy (PP), 31 acutely asphyxiated (AA) infants with 5-min Apgar <7 and/or UA-pH ≤7.05, and 38 control infants. Neurologic follow-up at 2 yr included Bayley score. Severe abnormality (S) was found in 4 PP and 5 AA infants, mild (M) in 12 and 6. High AVP was found only in normal AA infants (geom mean;95% conf: 303;146-633 pg/ml); M or S did not differ from controls (24;8-75). EP was high in PP infants regardless of outcome: normal (102;69-153), M (100;37-270), and S (84;19-378 mU/ml). AA infants with S outcome had higher EP (67;33-137) than M or normal or controls (38; 32-46). HX in PP infants was similar to controls. Normal AA infants had higher HX (24;17-33 μmol/l) than controls (12;10-16).

We conclude that neither AVP nor HX predicts brain damage. High EP after normal pregnancy, but not after preeclampsia, carries a risk for CP or death.

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PRODUCTION OF H_2O_2 BY STIMULATED PLATELETS. Domenico Del Principe,Adriana Menichelli,Stefano Di Giulio,Walter De Matteis,Massimo Giordani,Isabella Savini*,Gennaro Melino*,and Alessandro Finazzi-Agrò*. Depts.of Public Health and Cellular Biology,and of Experimental Medicine*,University of Rome "Tor Vergata", Rome,Italy.

Human platelets have been shown to release H_2O_2 when challenged with particulate stimuli.Here we report that platelets stimulated by thrombin also generate H_2O_2 inside,as detected by the peroxidation of the fluorogenic 2,7-dichlorofluorescein. The increase of the fluorescence is proportional to the amount of H_2O_2 generated by platelets.Under our experimental conditions,the fluorescence of intra-platelet 2,7-dichlorofluorescein is increased by 2-5 folds by stimulation with 1 U/ml of thrombin.Intracellular catalase reduces the extent of H_2O_2 production or its steady-state concentration.The ability of platelets to produce reduced,reactive oxygen radical species is noteworthy,since they have been found to play a role in pathological process,like the Kawasaki disease (Del Principe et al.,FEBS Lett.185:142,1985).

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OXYGEN RADICALS PRODUCE PULMONARY HYPERTENSION IN PIGS Jon Sanderud, Jarle Norstein and Ola D. Saugstad Inst. for Surgical Research Univ. of Oslo and Dept. of Pediatrics, The National Hospital, Oslo, Norway. The effect of oxygen radicals on the pulmonary circulation in pigs was studied. A bolus infusion of xanthine oxidase (XO) was given with or without hypoxanthine (Hx) into the right atrium. Pulmonary artery pressure (PAP) and pulmonary flow (PAQ) were measured continuously, and the pulmonary resistance (PR) was calculated. Three groups were studied: 1) Pigs given XO (1 U/kg). 2) Pigs given XO combined with Hx. 3) Pigs pretreated with indomethacin (7.5 mg/kg) before infusion of XO. Results: The table shows absolute differences from baseline values 20 minutes after XO was administered.

Group	PAP mm Hg	PAQ ml/kg/min	PR dyne sec cm^{-5}	p<
1 (n=6)	21.9 ± 4.3	-20.7 ± 13.3	1759 ± 671	0.001
2 (n=5)	29.2 ± 4.5	-14.0 ± 8.3	1570 ± 343	0.001
3 (n=6)	9.5 ± 8.7	- 5.6 ± 7.1	571 ± 352	NS

Infusion of XO both with and without Hx gave a significant increase in PAP and PR with PAQ decreasing concomitantly. Animals pretreated with indomethacin did not show any significant differences from baseline levels. This study demonstrates that XO potently constricts the pulmonary circulation in pigs. Since pretreatment with indomethacin only gives a blunted response, the effect may be mediated by the prostaglandin system.