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Iotrol, Iodixanol, and 2-Deoxy-D-Glucose Effects on Neural Tissue CO_2 Production

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In vivo and in vitro experiments have demonstrated that the myelographic agent metrizamide decreases neural tissue glucose metabolism whereas iohexol and iopamidol do not. This study compares the changes in slices of rat hippocampus CO_2 production caused by the nonionic dimers iotrol and iodixanol with the effects of metrizamide and 2-deoxy-D-glucose. After 6-hr incubations, 70-mmol/l concentrations of iotrol and iodixanol increased CO_2 production by 11 ± 20% and 31 ± 35%, respectively, as compared with the artificial CSF control medium. Metrizamide at 70 mmol/l and 2-deoxy-D-glucose at 35 mmol/l decreased CO_2 production by 32 ± 13% and 96 ± 1%, respectively.

The increases in CO_2 production with iotrol and iodixanol appear to indicate that these molecules have some effect on cell metabolism. The mechanism for the increase in CO_2 production could involve an effect on the glucose metabolic pathway or could be indirect via a mechanism that increases cell energy utilization. These in vitro effects have not been verified with in vivo experiments.

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In previous in vitro studies it has been established that the myelographic agent metrizamide inhibits hexokinase reactions [1, 2] and CO_2 production by rat hippocampus slices [3]. In vivo experiments in rabbits [4] and rats [5] have demonstrated a decrease in brain tissue 2-deoxy-D-glucose (2DG) uptake, further indicating an effect on glucose metabolism. In vitro studies of iohexol and iopamidol did not indicate an effect of these agents on either hexokinase or hippocampus slice CO_2 production [2, 6, 7]. The in vivo rabbit experiments also did not show an effect of iohexol on 2DG uptake [4]. These differences parallel the clinical experience that metrizamide produces more adverse effects than the new nonionic contrast media [8]. Although there is no proof that the clinical adverse effects of metrizamide are related to glucose metabolism, the similarity to the in vitro CO_2 production results lends support to the usefulness of the rat hippocampus model.

The purpose of these experiments was to examine the effects of two nonionic dimer contrast media, iotrol* and iodixanol,† on the production of CO_2 by using the rat hippocampus slice model previously used to test metrizamide, iopamidol, and iohexol. In addition, positive control experiments were performed with metrizamide and 2DG,‡ which is known to compete with glucose for the membrane glucose carrier and for the intracellular enzyme hexokinase.

Materials and Methods

Twenty-four adult Wistar rats were decapitated with a Harvard Apparatus small animal guillotine. The brain of each animal was quickly removed and sectioned in half along the midline. Each hemisphere was placed temporarily in room-temperature (22°C) 0.9% saline and the hippocampus was removed with surgical probes and blunt dissection. The hippocampus was transferred to filter paper saturated with oxygenated artificial CSF (see Table 1,

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* Iotrolan, Schering, Schering, Berlin, W. Germany.
† Nycomed, Olso, Norway.
‡ Sigma, St. Louis, MO.

TABLE 1: CSF Solution Compositions

	CSF (mmol/l)	CSF/CM (mmol/l)
Na^+	127.00	94.00
K^+	3.25	2.40
Mg^{++}	2.00	1.50
Ca^{++}	2.00	1.50
Cl^-	133.00	98.50
SO_4^{--}	2.00	1.50
H_2PO_4	1.25	0.90
Trizma HCl	25.00	25.00
Glucose	5.00	5.00
Contrast medium	–	70.00*

* 70 mmol/l is equivalent to 53 mg I/ml for dimers and 26.5 mg I/ml for the monomers.

Note.—CM = contrast medium.

control) and sectioned transverse to the long axis into 250-μm slices with a Sorvall TC-2 tissue sectioner. The 250-μm slices were placed in oxygenated control CSF at 37°C and separated from one another with artists' small brushes. Tissue slices from each hippocampus were divided evenly between the vials containing control and test CSF solutions (Table 1). Each vial contained approximately eight slices (approximately 0.5 mg of protein). The solution in each vial was "bubbled" with 95% oxygen and 5% carbon dioxide, sealed with a rubber sleeve stopper, and placed in a shaker bath at 37°C for a 4-hr incubation period.

The control and artificial test solutions in Table 1 were prepared by pipetting concentrated stock solutions of electrolytes, glucose, and contrast media into separate volumetric flasks and bringing the solutions to full volume with sterile distilled water. The concentrations of metrizamide, iotrol, and iodixanol were set at 70 mmol/l. These contrast media concentrations (26.5–53 mg I/ml) are well within the range of CSF concentrations observed after myelography. The final solutions had osmolalities of 306 mOsm/kg. At the completion of the 4-hr incubation period, 25 μl of a control CSF solution containing uniformly labeled 14C-D-glucose (American Radiolabeled Chemical, 38.5 μl/ml or 1.42 MBq/ml) was added to each of the vials. The solutions were then "bubbled" with 95% O_2 and 5% CO_2, sealed, and incubated for an additional 2 hr at 37°C in the shaker bath.

At the completion of the 2-hr incubation with labeled glucose the vials were removed from the bath and 1 ml of 2N hydrochloric acid solution was injected through each stopper to stop metabolism and to force the carbon dioxide into the gas phase. The CO_2 was collected by recirculating the gas trapped in each vial through 1 ml of 1 mol/l hyamine hydroxide in methanol for 15 min by using a multichannel pump (master flex drive #7567-50 with pump heads #7104-20 and .064 in. ID silicon tubing). Care was taken to avoid liquid transfer through the system and the system was rinsed with 1N hydrochloric acid before each use.

Aliquots of hyamine hydroxide containing the 14C-D-glucose were pipetted into 10 ml of Dimiscint[§] and the 14C activity counted with a Beckman LS 8100[‖] liquid scintillation counter. Background levels of activity were obtained from test vials containing all the ingredients except tissue samples. Gas collected from these vials contained a low level of activity (<4% of test), which was subtracted from all other measurements.

The tissue slices were removed from the CSF and hydrochloric acid solutions and placed in vials containing 1 ml of 1N sodium hydroxide solution. These vials were placed in a heater block at 40°C until the tissue was digested. An aliquot of each well-mixed solution was analyzed for protein content by using a commercial spectrophotometric assay.[¶] Preliminary studies demonstrated that the contrast media did not interfere with the protein assay.

The test solutions for examining the effects of 2DG were the same as in Table 1 except that 2DG was substituted at 0, 5, 7.5, 10, 17.5, 35, and 70 mmol/l for sodium chloride. All other procedures were the same as for the contrast media.

Specific activities of the bath solutions were determined and the CO_2 production was computed in units of dpm/μg protein and μmol/mg protein, assuming complete recovery of all CO_2. However, since we are interested in differences between the control and test solutions all results are reported as a percent change from control. Differences in CO_2 production were tested for significance by using Student's t test for unpaired samples. Means and standard deviations are presented in the text and figures.

Results

The changes in CO_2 production in the presence of iotrol, metrizamide, iodixanol, and 35 mmol/l 2DG are shown as a percent of control CO_2 production in Fig. 1 and Table 2. Metrizamide caused a 32 ± 13% reduction in CO_2 production ($p < .001$). Iodixanol caused a 31 ± 35% increase ($p < .001$), and iotrol an 11 ± 20% increase ($p < .02$). The positive control solution, 35 mmol/l 2DG, caused a 96 ± 1% decrease ($p < .0001$).

The concentration dependence of the effect of 2DG on CO_2 production is illustrated in Fig. 2. At concentrations less than or equal to 5.0 mmol/l, the 2DG had no significant effect. At concentrations greater than or equal to 10 mmol/l, there was a significant concentration-dependent decrease in CO_2 production.

Discussion

The 32% depression in CO_2 production caused by 70 mmol/l metrizamide in this study was greater than the 23% reduction observed previously [3]. At the same concentration, the two nonionic dimers, iotrol and iodixanol, caused increases in CO_2 production of 11 and 31%, respectively. These increases are significantly different from the decreases observed with metrizamide and 2DG ($p < .001$). In previous studies at the same concentrations iohexol and iopamidol did not cause significant changes in CO_2 production [6, 7]. The protocols of the previous experiments were identical except that the solutions contained a bicarbonate buffer instead of the Tris in the current solutions. Since the buffer concentrations were the same in control and test solutions for each experiment it seems unlikely that the change in buffers would cause a qualitative difference in results. The metrizamide and 2DG effects of the present study are within the range of effects observed in the previous studies.

The dimers iotrol and iodixanol have six iodine atoms per molecule; therefore, the iodine concentration of the iodixanol and iotrol was double that of metrizamide. At equal iodine concentrations to metrizamide the dimer nonionics might

[§] National Diagnostics, Somerville, NJ.

[‖] Beckman, Fullerton, CA.

[¶] BioRad Laboratories, Richmond, CA.

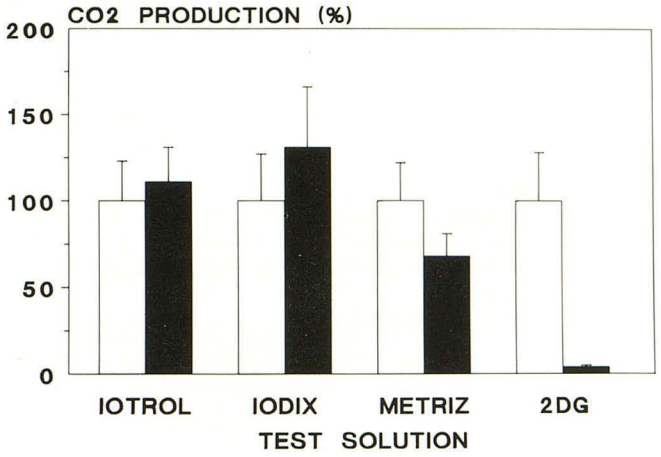

Fig. 1.—The effects of metrizamide (70 mmol/l), iodixanol (70 mmol/l), iotrol (70 mmol/l), and 2-deoxy-D-glucose on rat hippocampus CO_2 production are illustrated. Open bars represent control CSF solutions, shaded bars represent contrast media test solutions. Means and standard deviations are given.

TABLE 2: CO_2 Production (% Change from Control Mean)

Contrast Medium	Controls			Test Solutions			
	No.	Mean	SD	No.	Mean	SD	Concentration (mmol/l)
Iotrol	48	0	23	40	11	20	70
Iodixanol	32	0	27	21	31	35	70
Metrizamide	24	0	22	15	−32	13	70
2-deoxy-D-glucose	16	0	28	8	−96	1	35

Note.—SD = standard deviation.

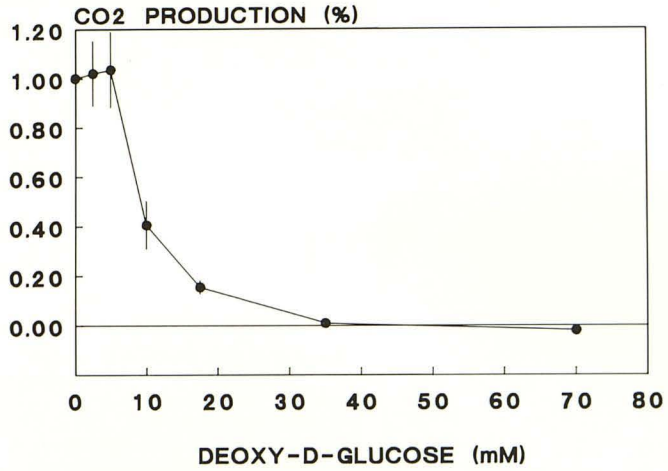

Fig. 2.—The interference of 2-deoxy-D-glucose on slices of rat hippocampus CO_2 production is strongly dependent on dose, and it is much greater than the effect of contrast media, as shown in this graph. Means ± standard deviation are given. Each point represents four experiments.

cause significantly smaller increases than were observed in this study.

The increase in CO_2 production with iodixanol is significantly greater than the increase with iotrol ($p < .01$). Since osmolality and electrolyte concentrations were essentially the same for the two solutions this difference would appear related to some difference in the two dimer molecules. The iotrol molecule for example has 12 hydroxyl groups compared with nine for iodixanol. These hydroxyl groups make the molecules more hydrophyllic and this is believed to decrease toxicity [9]. The four organic side chains of the iotrol molecule each contain four carbons and three hydroxyls, while the iodixanol side chains have three carbons and two hydroxyls. The larger, more hydrophyllic side chains of iotrol may more effectively shield the relatively hydrophobic iodine molecules. In principle these differences could influence the interactions of the molecules with cell membranes. However, there is no evidence that these differences in the molecules are related to the CO_2 production effects. There is also a small difference in the electrolyte concentration, since iotrol is commercially formulated with a small amount of sodium bicarbonate. The concentration of sodium bicarbonate in the test iotrol solution was less than 1 mmol/l and probably cannot account for this effect.

The 2DG molecule produced a large decrease in CO_2 production even at a concentration of 10 mmol/l, which was far below the 70 mmol/l concentration of the contrast media. This significant response to a known inhibitor of glucose metabolism indicates that the hippocampus slice model is appropriately sensitive to metabolic disturbances.

In the hippocampus slice CO_2 production model the optimal contrast medium should produce neither a decrease nor an increase in CO_2 production as compared with the control artificial CSF solutions. Either a decrease or increase must be interpreted as an effect of the contrast medium molecule on the neural cells. The decrease in CO_2 production seen with metrizamide indicates that metrizamide either directly inhibits glucose metabolism or somehow reduces the energy needs of the neural cells. The increase in CO_2 production seen with iotrol and iodixanol indicates a direct stimulation of glucose metabolism or an increase in the energy needs of the cells. It is known that electrical- or elevated-potassium-induced increases in neural activity and depolarization increase CO_2 production [10]. Drug-induced seizures are also known to increase CO_2 production [11]. Our current studies do not provide us with sufficient information to describe either mechanisms of action or the relative risks associated with either increased or decreased CO_2 production.

Extrapolation from these in vitro experiments to the clinical condition is difficult for a number of reasons. In our model we have examined only CO_2 production (or aerobic metabolism) and not anaerobic glycolysis. It is possible that ATP levels are not greatly affected. It is also possible that the effect on CO_2 production is not involved in the primary mechanisms for the neurotoxicity of myelographic contrast media. In vivo there are a much greater number of variables. For example, the in vitro model eliminates variations in blood delivery and greatly reduces diffusion distances for the contrast medium molecules. In vivo, the larger size of the dimers probably reduces the rate of their diffusion into the neural tissue as compared with the monomers. Additional information is clearly necessary to establish the relationship of these CO_2 effects to clinical toxicity.

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