

Short Communication

Dexmedetomidine-Induced Stimulation of Glutamine Oxidation in Astrocytes: A Possible Mechanism for Its Neuroprotective Activity

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Summary: Dexmedetomidine is a highly specific α_2 -adrenergic agonist, which is used clinically as an anesthetic adjuvant and in animal experiments has a neuroprotective effect during ischemia. The current study showed that dexmedetomidine enhances glutamine disposal by oxidative metabolism in astrocytes. This effect occurs at pharmacologically rel-

evant concentrations. It is exerted on α_2 -adrenergic receptors and not on imidazoline-preferring sites, and it is large enough to reduce the availability of glutamine as a precursor of neurotoxic glutamate. **Key Words:** Anoxia—Brain ischemia—Excitotoxicity—Glia—Glutamate—Glutamine.

Dexmedetomidine is a potent and highly specific α_2 -adrenergic agonist (Virtanen, 1989) used clinically as an anesthetic adjuvant (Aantaa et al., 1997; Venn et al., 1999). Doses of 1–100 $\mu\text{g}/\text{kg}$ have a neuroprotective effect during ischemia (Hoffman et al., 1991; Maier et al., 1993; Kuhmonen et al., 1997; Jolkkonen et al., 1999). With a molecular weight of 186 and a volume of distribution of 2–3 liter/kg (Scheinin et al., 1992; Muge et al., 1996), the dose range corresponds to plasma concentrations of 2.5–250 nmol/L.

It was originally assumed that all α_2 -adrenergic receptors are presynaptic and inhibit noradrenaline release. Although α_2 -adrenergic agonists have such an effect (Hong et al., 1992; Matsumoto et al., 1993; Gobert et al., 1998), compelling evidence for an additional localization on post-junctional target cells has appeared. The target cells include astrocytes (Ebersolt et al., 1981; Lee et al., 1998a,b). Stimulation of astrocytic α_2 -adrenergic receptors activates the phosphatidylinositol second messenger system (Enkvist et al., 1996) and increases free cytosolic calcium concentration, $[\text{Ca}^{2+}]_i$ (Salm and

MacCarthy, 1990; Zhao et al., 1992; Muyderman et al., 1997; Chen and Hertz, 1999). Clonidine, a less selective α_2 -adrenergic agonist, also increases glutamine uptake in astrocytes and its subsequent oxidative degradation (Huang and Hertz, 1994, 1995).

The importance of glutamine for glutamate-induced excitotoxic injury is illustrated by the observation that a decrease of the glutamine concentration in the culturing medium reduces anoxic death of cultured neurons (Goldberg et al., 1988; Huang et al. 1997). Oxidative degradation of glutamine occurs through pyruvate and lactate (Sonnewald et al., 1995; Schousboe et al., 1994). This is probably the main mechanism by which the brain disposes of excess glutamine and glutamate (Hertz et al., 1999, 2000). During anoxia this process is halted, but it can be re-established after reoxygenation (Pascual et al., 1998). In the current study it is shown that dexmedetomidine at pharmacologically relevant concentrations increases glutamine oxidation in astrocytes.

MATERIALS AND METHODS

Chemicals for tissue culture medium were from Sigma Chemical Company (St. Louis, MO, U.S.A.). Dexmedetomidine was donated by Orion Corporation Farnos (Turku, Finland). Idazoxan was bought from Research Biochemicals Inc.

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(Natick, MA, U.S.A.). L-[U-¹⁴C]glutamine was from NEN, Du Pont Canada Inc., Mississauga, Ontario. [1-¹⁴C]glutamine was donated by Drs. T. Tildon and H.R. Zielke, University of Maryland, MD, U.S.A.

Cultures of astrocytes were prepared as previously described (Juurlink and Hertz, 1992; Hertz et al., 1998). The neopallia, that is, the parts of the cerebral hemispheres above the lateral ventricles, were dissected out of brains from newborn Swiss mice and mechanically dissociated in a slightly modified Dulbecco's tissue culture medium (Juurlink and Hertz, 1992). The cell suspension was seeded in Falcon Primaria culture dishes and grown under a CO₂/air (5%/95%) atmosphere at 37°C. After 2 weeks the culturing was continued in the additional presence of 0.25 mmol/L dibutyryl cyclic adenosine monophosphate, which promotes morphologic and functional differentiation (Meier et al., 1991; Enkvist et al., 1996). The cultures were used after 3 to 6 weeks.

Production of labeled CO₂ from 0.2 mmol/L [U-¹⁴C]glutamine (0.3 µCi/ml) was measured during 60 minutes of incubation of individual cultures in an air-tight chamber (Yu et al., 1982). The cells were incubated in modified Dulbecco's medium, prepared without glucose to prevent the production of labeled CO₂ reflecting an exchange between labeled glutamate and non-labeled α-ketoglutarate formed from glucose. At the end of the experiments the medium was acidified by injection of acetic acid, converting bicarbonate to CO₂. During subsequent incubation for 60 minutes on a hotplate (60°C), which created air currents within the chamber, all CO₂ was quantitatively trapped into 10% tetramethylammonium hydroxide which had been injected into a presuspended beaker. Accumulated radioactivity was determined in a liquid scintillation counter, and glutamine oxidation rates/mg protein were calculated on the basis of the specific activity of the medium and the protein content in the culture, as measured by the Lowry technique (Lowry et al., 1951).

A few experiments were carried out using [1-¹⁴C]glutamine instead of L-[U-¹⁴C]glutamine. This provided a quantitatively more correct estimate of glutamine disposal because the carbon in the C-1 position is the first to be oxidized. Under control conditions the rate of ¹⁴CO₂ production from [1-¹⁴C]glutamine was 2.7 ± 0.3 times higher (n = 6) than that from [U-¹⁴C]glutamine.

RESULTS

The apparent rate of ¹⁴CO₂ production under control conditions was 0.24 ± 0.01 nmol/min·mg protein. It increased by ~25% by exposure to either 70 nmol/L or 25 µmol/L dexmedetomidine (Table 1). The apparent control rate and the stimulation of 0.06 nmol/min·mg protein are nominal values that underestimate glutamine oxidation for two reasons: (1) not all 5 carbon atoms of uniformly labeled glutamine are oxidized within the 1-hour incubation period, and (2) the specific activity of intracellular [U-¹⁴C]glutamine is lower than that in the medium (Huang et al., 1995). The first of these sources of error can be corrected by aid of the ratio of 2.7 between rate of ¹⁴CO₂ production from [1-¹⁴C]glutamine and [U-¹⁴C]glutamine (see Methods). Using this ratio as a correction factor, the dexmedetomidine-induced increase in rate of glutamine disposal can be estimated to -0.2 nmol/min·mg protein. This value remains an

TABLE 1. Rates of ¹⁴CO₂ production from 0.2 mmol/L L-[U-¹⁴C] glutamine in primary cultures of mouse astrocytes

Inhibitor	Dexmedetomidine concentration		
	0	70 nmol/L	25 µmol/L
None	100 ± 2.6 (12)*	126.3 ± 10 (6)	125.0 ± 6.0 (12)†
Yohimbine‡	107.4 (2)		97.8 ± 7.4 (7)
Idazoxan‡	92.1 (2)		115.0 ± 8.2 (7)

Rates during exposure to 70 nmol/L and 25 µmol/L dexmedetomidine and to 25 µmol/L dexmedetomidine in the additional presence of yohimbine or idazoxan.

* All values are expressed as percentages of the control value in the same experiment in the absence of any drug. Values are averages ± SEM with number of individual experiments shown in parentheses.

† The effect of 25 µmol/L dexmedetomidine is statistically significantly different from both the control value and the value in the additional presence of yohimbine in ANOVA plus Fisher PLSD test for multiple comparisons.

‡ 100 µmol/L.

underestimate because extracellular, not intracellular, specific activity was used for the determination (Zielke et al., 1996). However, because of possible intracellular compartmentation of glutamine (Schousboe et al., 1994), no attempt was made to correct this source of error.

The dexmedetomidine-induced stimulation of U-[¹⁴C]glutamine oxidation was exerted on α₂-adrenergic receptors as shown by its inhibition by yohimbine, an α₂ antagonist (Table 1), but not by idazoxan, an inhibitor of the imidazoline binding site (Bylund, 1995). The small, insignificant reduction of dexmedetomidine-stimulated ¹⁴CO₂ by idazoxan reflects that idazoxan, on its own, caused approximately a 10% reduction in the rate of ¹⁴CO₂ formation.

DISCUSSION

Dexmedetomidine *in vivo* exerts other actions than inhibiting noradrenaline release as proven by the fact it has a sedative effect and potentiates anesthetics in animals depleted for noradrenaline (Segal et al., 1988; Riekinen et al., 1993), an effect exerted within the central nervous system (Doze et al., 1989). The current results suggest that some of dexmedetomidine's actions are exerted on astrocytic α₂-adrenergic receptors. This is consistent with a dexmedetomidine-induced increase in astrocytic [Ca²⁺]_i (Zhao et al., 1992; Enkvist et al., 1996; Chen et al., 2000).

In heart cells, an increase in [Ca²⁺]_i resulting from adrenergic stimulation leads to uptake of K⁺ in mitochondria and swelling of the mitochondrial matrix, which in turn stimulates glutaminase activity (Halestrap, 1989). The same mechanisms may operate in astrocytes because they display considerable glutaminase activity (Hogstad et al., 1988; Aoki et al., 1991; Wurdig and Kugler, 1991), which is increased by clonidine (Huang and Hertz, 1994, 1995). Moreover, α₂-adrenergic agonists have a direct effect on oxidation of α-ketoglutarate

(Subbarao and Hertz, 1991) which, combined with an increased rate of formation of glutamate from glutamine, could explain the increase in glutamine oxidation seen in the current study.

Dexmedetomidine concentration of 70 nmol/L, which stimulated glutamine oxidation by 25%, is within the plasma concentration range expected after administration of dexmedetomidine as a neuroprotectant. The effect is large enough that it may affect glutamine content in the brain, a novel mechanism of action for a neuroprotectant drug. Net synthesis of glutamate and glutamine from glucose depends upon pyruvate carboxylation, which in the brain occurs at a rate 10–15% of total TCA cycle activity (Lapidot and Gopher, 1994; Aureli et al., 1997; Gruetter et al., 1998). Based upon this information and a glucose utilization rate in the mouse brain of 60–70 $\mu\text{mol}/\text{min}$ per 100 g (Wree et al., 1989), it can be estimated that the maximum rate of net glutamate/glutamine synthesis from glucose in the mouse brain amounts to 1.2–2.1 nmol/min-mg protein (Hertz et al., 1999). A dexmedetomidine-induced increase of glutamine disposal of at least 0.2 nmol/min-mg protein may cause a substantial change in the balance between formation and disposal of glutamine, thus reducing the availability of glutamine as a glutamate precursor. This concept is consistent with the observation by Talke and Bickler (1996) that glutamate release from hippocampal slices during hypoxia is decreased by dexmedetomidine.

REFERENCES

- Aantaa R, Jaakola ML, Kallio A, Kanto J (1997) Reduction of the minimum alveolar concentration of isoflurane by dexmedetomidine. *Anesthesiology* 86:1055–1060
- Aoki C, Kaneko T, Starr A, Pickel VM (1991) Identification of mitochondrial and non-mitochondrial glutaminase within select neurons and glia of rat forebrain by electron microscopic immunocytochemistry. *J Neurosci Res* 28:531–548
- Aureli T, Di Cocco ME, Calvani M, Conti F (1997) The entry of [^{14}C]glucose into biochemical pathways reveals a complex compartmentation and metabolite trafficking between glia and neurons: A study by ^{13}C -NMR spectroscopy. *Brain Res* 765:218–227
- Bylund DB (1995) Pharmacological characteristics of alpha-2 adrenergic receptor subtypes. *Ann N Y Acad Sci* 763:1–7
- Chen Y, Hertz L (1999) Noradrenaline effects on pyruvate decarboxylation-correlation with calcium signaling. *J Neurosci Res* 58:599–606
- Chen Y, Zhao Z, Code WE, Hertz L (2000) Correlation between dexmedetomidine-induced biphasic increases in free cytosolic calcium concentration and in energy metabolism in astrocytes. *Anesth Analg*. (in press)
- Doze VA, Chen BX, Maze M (1989) Dexmedetomidine produces a hypnotic-anesthetic action in rats via activation of central alpha₂ receptors. *Anesthesiology* 71:75–79
- Ebersolt C, Perez M, Bockaert J (1981) Alpha 1 and alpha 2 adrenergic receptors in mouse brain astrocytes from primary cultures. *J Neurosci Res* 6:643–652
- Enkvist MO, Hamalainen H, Jansson CC, Kukkonen JP, Hautala R, Courtney MJ, Akerman KE (1996) Coupling of astroglial alpha₂-adrenoreceptors to second messenger pathways. *J Neurochem* 66:2394–2401
- Gobert A, Rivet JM, Audinot V, Newman-Tancredi A, Cistarelli L, Millan MJ (1998) Simultaneous quantification of serotonin, dopamine and noradrenaline levels in single frontal cortex dialysates of freely moving rats reveals a complex pattern of reciprocal auto- and heteroreceptor-mediated control of release. *Neuroscience* 84:413–429
- Goldberg MP, Monyer H, Choi DW (1988) Hypoxic neuronal injury in vitro depends on extracellular glutamine. *Neurosci Lett* 94:52–57
- Gruetter R, Seaquist ER, Kim S, Ugurbil K (1998) Localized in vivo ^{13}C NMR of glutamate metabolism in the human brain: initial results at 4 tesla. *Dev Neurosci* 20:380–388
- Halestrap AP (1989) The regulation of the matrix volume of mammalian mitochondria in vivo and in vitro and its role in the control of mitochondrial metabolism. *Biochim Biophys Acta* 973:355–382
- Hertz L, Peng L, Lai JCK (1998) Functional studies in cultured astrocytes. *Methods: A Companion to Methods in Enzymology* 16:293–310
- Hertz L, Dringen R, Schousboe A, Robinson S (1999) Astrocytes: Glutamate producers for neurons. *J Neurosci Res* 57:417–428
- Hertz L, Yu ACH, Kala G, Schousboe A (2000) Neuronal-astrocytic and cytosolic-mitochondrial metabolite trafficking during brain activation, hyperammonemia and energy deprivation. *Neurochem Int* (in press)
- Hoffman WE, Kochs E, Werner C, Thomas C, Abrecht RF (1991) Dexmedetomidine improves neurologic outcome from incomplete ischemia in the rat. Reversal by the alpha-2-adrenergic antagonist atipamezole. *Anesthesiology* 75:328–332
- Hogstad S, Svenneby G, Torgner IA, Kvamme E, Hertz L, Schousboe A (1988) Glutaminase in neurons and astrocytes cultured from mouse brain: kinetic properties and effects of phosphate, glutamate and ammonia. *Neurochem Res* 13:383–388
- Hong M, Milne B, Loomis C, Jhamandas K (1992) Stereoselective effects of central alpha-2-adrenergic agonist medetomidine on in vivo catechol activity in the rat rostral ventrolateral medulla. *Brain Res* 592:163–169
- Huang R, Hertz L (1994) Clonidine enhances astrocytic glutamine uptake by authentic α_2 -adrenoceptor stimulation. *Neuroreport* 5:632–634
- Huang R, Hertz L (1995) Noradrenaline induced stimulation of glutamine metabolism in primary cultures of astrocytes. *J Neurosci Res* 41:677–683
- Huang R, Scothocka E, Hertz L (1997) Cell culture studies of the role of elevated extracellular glutamate and K^+ in neuronal cell death during and after anoxia/ischemia. *Neurosci Biobehav Rev* 21:129–134
- Jolkonen J, Puurunen K, Koistinaho J, Kauppinen R, Haapilina A, Nieminen L, Sivenius J (1999) Neuroprotection by the alpha-2-adrenoceptor agonist, dexmedetomidine, in rat focal cerebral ischemia. *Eur J Pharmacol* 372:31–36
- Jurilink BHJ, Hertz L (1992) Astrocytes. In: *Neuroanatomy* (Boulton AA, Baker GB, Wals W, eds), New York: Humana, Clifton, pp 269–321
- Kultmonen J, Pokorny J, Miettinen R, Haapilina A, Jolkonen J, Riekkinen P, Sivenius J (1997) Neuroprotective effects of dexmedetomidine in the gerbil hippocampus after transient global ischemia. *Anesthesiology* 87:371–377
- Lapidot A, Gopher A (1994) Cerebral metabolic compartmentation-estimation of glucose flux via pyruvate carboxylase/pyruvate dehydrogenase by ^{13}C NMR isotopomer analysis of D-[U- ^{13}C] glucose metabolites. *J Biol Chem* 269:27198–27208
- Lee A, Rosin DL, Van Bockstaele EJ (1998a) Alpha₂-adrenergic receptors in the rat nucleus locus coeruleus: subcellular localization in catecholaminergic dendrites, astrocytes, and presynaptic axon terminals. *Brain Res* 795:157–169
- Lee A, Rosin DL, Van Bockstaele EJ (1998b) Ultrastructural evidence for prominent postsynaptic localization of alpha₂-adrenergic receptors in catecholaminergic dendrites in the rat nucleus locus coeruleus. *J Comp Neurol* 394:218–229
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265–275
- Maier C, Steinberg GK, Sun GH, Zhi GT, Maze M (1993) Neuroprotection by the alpha-2-adrenoceptor agonist dexmedetomidine in a focal model of cerebral ischemia. *Anesthesiology* 79:306–312
- Matsumoto M, Zornow MH, Rabin BC, Maze M (1993) The alpha₂-adrenergic agonist, dexmedetomidine, selectively attenuates ischemia

- emia-induced increases in striatal norepinephrine concentrations. *Brain Res* 627:325-329
- Meier E, Hertz L, Schousboe A (1991) Neurotransmitters as developmental signals. *Neurochem Int* 19:1-15
- Muge DK, Chambers JP, Livingston A (1996) Single dose pharmacokinetics of dexmedetomidine in sheep. *J Vet Pharmacol Ther* 19:109-112
- Muyderman H, Hansson E, Nilsson M (1997) Adrenoceptor-induced changes of intracellular K^+ and Ca^{2+} in astrocytes and neurons in rat cortical primary cultures. *Neurosci Lett* 238:33-36
- Pascual JM, Carceller F, Roda JM, Cerdan S (1998) Glutamate, glutamine and GABA as substrates for the neuronal and glial compartments after focal cerebral ischemia in rats. *Stroke* 29:1048-1057
- Riekkinen P Jr, Lammintausta R, Ekonsalo T, Sirvio J (1993) The effects of alpha 2-adrenoceptor stimulation on neocortical EEG activity in control and 6-hydroxydopamine dorsal noradrenergic bundle-lesioned rats. *Eur J Pharmacol* 238:263-272
- Salm AK, McCarthy KD (1990) Norepinephrine-evoked calcium transients in cultured cerebral type-1 astroglia. *Glia* 3:529-538
- Scheinin H, Karhuvaara S, Olkkola KT, Kallio A, Anttila M, Vuoriohto L, Scheinin M (1992) Pharmacodynamics and pharmacokinetics of intramuscular dexmedetomidine. *Clin Pharmacol Ther* 52:537-546
- Schousboe A, Westergaard N, Sonnewald U, Petersen S, Huang R, Hertz L (1994) Glutamate and glutamine metabolism and compartmentation in astrocytes. *Dev Neurosci* 15:359-366
- Segal JS, Vickery RG, Walton JK, Doze VA, Maze M (1988) Dexmedetomidine diminishes halothane anesthetic requirements in rats through a postsynaptic alpha 2 adrenergic receptor. *Anesthesiology* 69:818-823
- Sonnewald U, Westergaard N, Jones P, Taylor A, Bachelard HS, Schousboe A (1995) Metabolism of $[U-^{14}C]$ glutamine in cultured astrocytes studied by NMR spectroscopy: first evidence of astrocytic pyruvate recycling. *J Neurochem* 67:2566-2572
- Subbarao KV, Hertz L (1991) Stimulation of energy metabolism in astrocytes by adrenergic agonists. *J Neurosci Res* 28:399-405
- Talke P, Bickler PE (1996) Effects of dexmedetomidine on hypoxia-evoked glutamate release and glutamate receptor activity in hippocampal slices. *Anesthesiology* 85:551-557
- Venn KM, Bradshaw CJ, Spencer R, Brealey D, Caudwell E, Naughton C, Vedio A, Singer M, Feneck R, Treacher D, Willatts SM, Grounds RM (1999) *Anaesthesia* 54:1136-1142
- Virtanen R (1989) Pharmacological profiles of medetomidine and its antagonist, atipamezole. *Acta Vet Scand Suppl* 85:29-37
- Wree A, Beck T, Bielenberg GW, Schleicher A, Zilles K (1989) Local cerebral glucose utilization in the autoimmune New Zealand black (NZB) mouse. *Histochemistry* 92:343-348
- Wurdig S, Kugler P (1991) Histochemistry of glutamate metabolising enzymes in the rat cerebellar cortex. *Neurosci Lett* 130:165-168
- Yu ACH, Schousboe A, Hertz L (1982) Metabolic fate of (^{14}C) -labelled glutamate in astrocytes. *J Neurochem* 39:954-966
- Zhao Z, Code WE, Hertz L (1992) Dexmedetomidine, a potent and highly specific alpha₂ agonist, evokes cytosolic calcium surge in astrocytes but not in neurons. *Neuropharmacology* 31:1077-1079
- Zielke HR, Tildon RT, Zielke CL (1996) Use of intracellular versus extracellular specific activities for calculation of glutamine metabolism in astrocytes: effects of dibutyryl cyclic AMP. *Dev Neurosci* 18:224-230