ELSEVIER

Cerebral Metabolic Responses to Clomipramine Are Greatly Reduced Following Pretreatment with the Specific Serotonin Neurotoxin Para-Chloroamphetamine (PCA)

A 2-Deoxyglucose Study in Rats

Ulderico Freo, M.D., Pietro Pietrini, M.D., Ph.D., Gilberto Pizzolato, M.D., Maura Furey, Ph.D., Antonio Merico, M.D., Susanna Ruggero, Ph.D., Mauro Dam, M.D., and Leontino Battistin, M.D.

To determine if reported reductions of regional cerebral metabolic rates for glucose (rCMRglc) induced by the tryciclic antidepressant clomipramine (CMI) (10 mg/kg) are due to a presynaptic action on serotonin (5-HT) terminals, 3-month-old Fischer-344 rats were given parachloroamphetamine (PCA), a serotonin neurotoxin. rCMRglc was measured 3 weeks later in 55 brain regions after the administration of saline or CMI using the quantitative autoradiographic [^{14}C]2-deoxyglucose procedure. PCA alone increased rCMRglc in the visual cortex. CMI alone reduced rCMRglc in 18 (33%) of the studied regions, including telencephalic, diencephalic, limbic, and brain stem areas. In PCA-lesioned rats, metabolic responses to CMI (10 mg/kg) were greatly reduced, and significant rCMRglc decreases were observed only in 4 (7%) of the brain areas, including the hippocampus and raphe nuclei. Abolition by PCA of the metabolic responses to CMI confirms that CMI, at the dose studied, reduces rCMRglc via a presynaptic mechanism, likely the 5-HT reuptake sites.
[Neuropsychopharmacology 13:215–222, 1995]

KEY WORDS: *Deoxyglucose; Clomipramine; Serotonin; Antidepressants; Amphetamine; Rat*

Clomipramine (CMI) is a tryciclic compound with marked antidepressive (Hall and Ogren 1981) and antiobsessional (Murphy and Pigott 1990) properties. Recent clinical trials indicated that CMI is also effective in treating panic attacks (Feet and Gotestam 1994; Modigh et al. 1992), phobic disorders (Hoffart et al. 1993), and premenstrual syndrome (Sundblad et al. 1993).

In vitro, CMI binds to different receptor types (e.g., dopaminergic D_2, histaminergic H_1, adrenergic α_1, and serotoninergic 5-HT_2 receptors) (Hall and Ogren 1981) and inhibits norepinephrine (Thomas and Jones 1977) and serotonin (5-HT) reuptake (Carlsson et al. 1969; Fuller and Wong 1977). In experimental animals, acutely administered CMI increases brain 5-HT concentrations

From the Clinica delle Malattie Nervose e Mentali (UF, GP, AM, SR, MD, LB), Via Giustiniani 5, I-35100 Padova, Italy; Clinica Psichiatrica (PP), Via Roma 67, I-56100, Pisa, Italy and the Laboratory of Neurosciences, NIA, NIH (MF), 9000 Rockville Pike, MD 20892, USA.

Address correspondence to: Ulderico Freo, M.D., Laboratory of Neurosciences, Bldg. 10, Room 6C414, National Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20892.

Received September 12, 1994; revised February 17, 1995; accepted March 7, 1995.

Published 1995 by Elsevier Science Inc.
655 Avenue of the Americas, New York, NY 10010

0893-133X/95/$0.00
SSDI 0893-133X(95)00053-G

and corticosterone plasma levels, while chronically administered CMI decreases brain 5-HT concentrations, reuptake and release, 5-HT_2 receptor number (Peroutka and Snyder 1980), and neuroendocrine responses to 5-HT agonists (Wozniak et al. 1989). Therapeutic actions of CMI have been attributed most frequently to its effects on the 5-HT system (Modigh et al. 1992; Murphy 1990; Murphy and Pigott 1990; Sundblad et al. 1993).

Recently, we reported that CMI decreases regional cerebral metabolic rates for glucose (rCMRglc) (Freo et al. 1993), a direct index of neuronal activity (Sokoloff et al. 1977) in areas with high densities of 5-HT reuptake sites (e.g., raphe nuclei and superior colliculus) (De Souza and Kuyatt 1987) with a time course that paralleled its effects on brain extracellular 5-HT (Carboni and Di Chiara 1989). Based on these findings we proposed that CMI may reduce rCMRglc specifically by blocking 5-HT reuptake. The aim of the present study was to test this hypothesis by determining whether the cerebral metabolic responses to CMI could be abolished by prior destruction of 5-HT presynaptic elements. Using the [^{14}C]2-deoxyglucose ([^{14}C]DG) technique (Sokoloff et al. 1977; Sokoloff 1982), rCMRglc responses to CMI were measured in control rats and in animals in which 5-HT terminals had been previously destroyed by administration of parachloroamphetamine (PCA), a selective 5-HT neurotoxin.

MATERIALS AND METHODS

Materials

Experiments were performed in male 3-month-old Fischer-344 rats purchased from Charles River Italia (Como, Italy). [^{14}C]2-Deoxy-D-glucose was obtained from New England Nuclear (Boston, MA), clomipramine hydrochloride from Research Biochemicals (Natick, MA), and p-chloroamphetamine from Sigma (St. Louis, MO). All drugs were dissolved in saline.

Drug Treatments

Rats were pretreated with PCA (10 mg/kg IP), twice, 24 hours apart, or with two saline injections. After pretreatment, all rats were maintained at 15°C ambient room temperature for 8 hours to protect them against transient hyperthermia produced by PCA.

Twenty-one days later, groups of five to six rats received saline or chlomipramine (10 mg/kg IP) as follows (times are relative to [^{14}C]DG administration; see Table 1).

Dose and time points were based on a previous study in which the time course and relation to dose of CMI metabolic effects were determined (Freo et al. 1993).

Table 1. Experimental Groups

Group	*n*	Pretreatment	Treatment
1	6	Saline	Saline, −120 minutes
2	5	Saline	CMI, −120 minutes
3	6	PCA	Saline, −120 minutes
4	6	PCA	CMI, −120 minutes

Abbreviations: CMI = clomipramine, PCA = parachloroamphetamine.

Biochemical Assay

Separate groups of animals were injected with saline or PCA (10 mg/kg IP), twice, 24 hours apart. Twenty-one days later they were decapitated between 11:00 A.M. and 12:00 P.M., and their brains were dissected. The neocortex, hippocampus, striatum, and brain stem were removed and stored at −70°C until assayed. The concentrations of serotonin and 5-hydroxyindoleacetic acid were determined with high-pressure liquid chromatography (Mefford 1981). In brief, frozen tissue samples were weighed and transferred to 1.5-ml Eppendorf tubes containing 100 µl 0.1 N perchloric acid and 50 µl 10^{-6} M N-methyl-serotonin. Samples were sonicated on ice for 1 minute (Heat Systems, model W-300 fitted with microtip) at a setting of 3. The mixture then was centrifuged at 12,000 g for 2 minutes, and the clear supernatant was removed. Sample was applied to the column using a Gilson model 231/401 sample injector (Thomson Instruments, Springfield, VA) fitted with a 50-µl loop.

rCMRglc

Under inhaled halothane (1.5%) anesthesia, polyethylene catheters were inserted into the right femoral artery and vein. The incision then was infiltrated with 0.2 ml lidocaine (1%) and closed with wound clips. Animals were restrained in a plaster cast loosely applied to the lower abdomen. This restraint has no effects on rCMRglc measured with [^{14}C]DG technique (Soncrant et al. 1988). Body temperature was kept between 35.5 and 37°C by a rectal thermoprobe connected to a thermostatic device (Indicating Controller, model 73ATA, Yellow Springs, OH) that activated a heating element when temperature fell below 35.5°C.

At least 4 hours after anesthesia, [^{14}C]DG was injected by rapid IV bolus, and 11 timed arterial blood samples were collected. Samples were centrifuged immediately; plasma radioactivity (Model LS9000 Liquid Scintillation Spectrometer, Beckman Instruments, Irvine, CA) and glucose concentrations (Glucose Analyzer II, Beckman, Irvine, CA) were determined. Rats were killed 45 minutes after [^{14}C]DG administration by an IV overdose of sodium pentobarbital (60 mg in 1 ml). The brains were removed rapidly and frozen in methylbutane at −55°C, then stored at −70°C.

Coronal sections, 20 μm thick, were made in a cryostat (Bright Model 5030, Hacker Instruments, Fairfield, NJ) at −18°C and dried immediately on a hot plate. Autoradiographs were obtained by exposing brain sections to Kodak SB-5 x-ray films, together with methylmethacrylate standards of known ^{14}C concentration (Amersham, Arlington Heights, IL). Densitometry of autoradiograms was performed with an image analyzer (MCID Image Analyzer, Image Res. Inc., Ontario, Canada). Six separate determinations of optical density were performed in both left and right sides of the brain, and the means were averaged. Brain regions were defined by comparison of autoradiographic sections with rat brain atlases (Paxinos and Watson 1982) and, where necessary, with cresyl violet-stained sections obtained from slices adjacent to those used for autoradiography. rCMRglc was calculated from brain and plasma radioactivity and plasma glucose concentrations, using equations and constants given by Sokoloff et al. (1977).

Statistical Analysis

Physiological parameters (measured before and at various time points after drug administration) and brain concentrations of 5-HT and 5-hydroxyindoleacetic acid of PCA-lesioned groups were compared to the mean of respective control groups by Dunnett's *t* test (Miller 1966).

rCMRglc values were analyzed for statistical significance by one-way analysis of variance and Bonferroni multiple comparison test. rCMRglc values of groups 2 (saline-pretreated, CMI-treated) and 3 (PCA-pretreated, saline-treated) were compared to those of group 1. rCMRglc values of group 4 (PCA-pretreated, CMI-treated) were compared to those of group 3.

RESULTS

Physiological Parameters

In control animals before and after [^{14}C]DG administration mean arterial blood pressure, heart rate, body temperature, and plasma glucose concentrations were similar to those reported previously (Freo et al. 1991; Pizzolato et al. 1985; Soncrant et al. 1985). These parameters were not altered ($p < .05$) by pretreatment with PCA or by administration of CMI (10 mg/kg).

Brain Neurochemistry

Brain concentration of serotonin and 5-hydroindolacetic acid determined 21 days after PCA administration were markedly reduced (see Table 2). Serotonin and 5-hydroxyindolacetic acid declined by 76% ($p < .01$) in the brain stem and by more than 90% ($p < .01$) in the neocortex, striatum, and hippocampus of PCA-pretreated animals.

Regional Cerebral Metabolic Rates for Glucose

Values of rCMRglc in saline and CMI animals, previously pretreated with saline or PCA are presented in Table 3. Control values of rCMRglc were similar to those reported in the literature for awake young rats (Freo et al. 1991; Pizzolato et al. 1985; Soncrant et al. 1985). PCA pretreatment increased rCMRglc in the visual cortex only.

In saline-pretreated rats, CMI reduced rCMRglc in 18 (33%) of the brain regions, including the dorsal and median raphe nuclei, superficial layer of superior colliculus, lateral septum, the areas with the highest concentrations of 5-HT reuptake sites (De Souza and Kuyatt, 1987). PCA pretreatment markedly attenuated CMI rCMRglc effects (Figure 1) that persisted only in hippocampal areas and in the raphe nuclei (4 regions, 7%).

DISCUSSION

PCA produces long-lasting degeneration of 5-HT fibers and reductions of presynaptic 5-HT markers (e.g., 5-HT and 5-hydroxyindoleacetic acid concentrations, tryptophan hydroxylases activity, and [^{3}H]5-HT synaptosomal uptake). In this study PCA pretreatment reduced 5-HT and 5-hydroxyindolacetic acid by 76 to 95%,

Table 2. Brain Concentrations of Serotonin and 5-Hydroxyindoleacetic Acid 21 Days after Saline or PCA Pretreatment

	Serotonin (μg/g)		5-Hydroxyindoleacetic Acid (μg/g)	
	Saline	PCA	Saline	PCA
Neocortex	362 ± 20	24 ± 6[a]	162 ± 8	24 ± 3[a]
Hippocampus	476 ± 32	45 ± 7[a]	501 ± 25	62 ± 9[a]
Striatum	963 ± 65	84 ± 17[a]	487 ± 32	48 ± 7[a]
Brain stem	689 ± 89	165 ± 65[a]	378 ± 56	95 ± 6[a]

Mean ± SEM, six animals per group. PCA-pretreated groups were compared to saline-pretreated groups by Dunnett's *t* test.
[a] $p < .01$.

Table 3. Effects of Pretreatment with Saline or Parachloroamphetamine (PCA) on rCMRglc Responses to Clomipramine (CMI) in Awake Fischer-344 Rats

	Pretreatment rCMRglc (*μmol/100 g/min*)			
	Saline		PCA	
Treatment	**Saline**	**CMI**	**Saline**	**CMI**
Cerebral cortex (layer IV)				
Frontal (area 10)	119 ± 4	100 ± 6*	113 ± 5	96 ± 5
Frontal (area 8)	132 ± 2	101 ± 3*	107 ± 3	99 ± 4
Precentral medial	144 ± 10	130 ± 10	120 ± 7	112 ± 6
Motor	124 ± 5	106 ± 9	108 ± 6	97 ± 6
Somatosensory	138 ± 7	119 ± 8	119 ± 8	107 ± 5
Pyriform (area 51b)	85 ± 1	82 ± 7	81 ± 4	77 ± 4
Auditory	162 ± 2	142 ± 9	169 ± 9	145 ± 7
Visual	103 ± 4	85 ± 2**	134 ± 4**	122 ± 4
Olfactory	111 ± 7	117 ± 10	117 ± 6	98 ± 4
Basal ganglia				
Caudate				
Mediolateral	122 ± 5	109 ± 9	108 ± 8	102 ± 7
Mediomedial	120 ± 4	98 ± 4**	101 ± 6	88 ± 6
Globus pallidus	59 ± 3	54 ± 2	51 ± 2	44 ± 2
Substantia nigra				
Pars reticularis	47 ± 4	49 ± 4	49 ± 3	46 ± 2
Pars compacta	69 ± 5	68 ± 4	68 ± 4	63 ± 4
Thalamus and subthalamus				
Thalamus				
Anteroventral n	113 ± 7	104 ± 10	112 ± 5	95 ± 8
Anteromedial n	113 ± 10	99 ± 10	98 ± 10	101 ± 6
Interanteromedial n	124 ± 9	110 ± 5	114 ± 5	99 ± 7
Ventroposteromedial n	99 ± 3	91 ± 8	91 ± 4	87 ± 4
Subthalamic n	92 ± 3	92 ± 9	75 ± 5	74 ± 5
Lateral geniculate	86 ± 4	68 ± 2*	75 ± 7	72 ± 4
Medial geniculate	131 ± 5	111 ± 8	134 ± 11	113 ± 8
Lateral habenula				
Medial part	73 ± 6	80 ± 2	77 ± 7	74 ± 3
Lateral part	145 ± 8	135 ± 3	124 ± 6	124 ± 7
Hypothalamus and preoptic areas				
Hypothalamus				
Lateral n	70 ± 3	60 ± 6	66 ± 5	58 ± 3
Paraventricular n	62 ± 4	58 ± 7	52 ± 4	49 ± 3
Mammillary nuclei				
Medial	110 ± 8	104 ± 9	92 ± 6	83 ± 4
Lateral	110 ± 6	111 ± 9	93 ± 7	90 ± 8
Preoptic areas				
Medial	40 ± 3	39 ± 2	41 ± 3	39 ± 5
Lateral	64 ± 4	63 ± 3	65 ± 4	61 ± 3
Magnocellular	94 ± 10	87 ± 8	89 ± 5	83 ± 4
Limbic system				
Amygdala				
Medial	39 ± 3	39 ± 1	41 ± 1	39 ± 5
Basolateral	98 ± 6	77 ± 4**	84 ± 3	75 ± 4
Cortical	91 ± 8	78 ± 8	79 ± 8	68 ± 4
Hippocampus (pyramidal layer)				
Dorsal CA1	57 ± 3	51 ± 4	55 ± 3	51 ± 2
CA3	63 ± 4	59 ± 6	66 ± 2	56 ± 3
Dentate gyrus	61 ± 4	52 ± 3*	57 ± 3	47 ± 3
Ventral CA1	58 ± 5	47 ± 1*	58 ± 3	42 ± 2##
CA3	71 ± 4	59 ± 2*	64 ± 3	52 ± 3#
Dentate gyrus	69 ± 5	52 ± 1**	59 ± 2	53 ± 3
Lateral septum	54 ± 8	43 ± 1*	53 ± 5	47 ± 2

(*continued*)

Table 3. *(continued)*

	Pretreatment rCMRglc (*μmol/100 g/min*)			
	Saline		PCA	
Treatment	**Saline**	**CMI**	**Saline**	**CMI**
Brain stem				
Superior colliculus				
Superficial layer	79 ± 4	60 ± 1**	77 ± 5	72 ± 2
Deep layer	94 ± 4	75 ± 1**	79 ± 6	70 ± 4
Oculomotor complex	90 ± 3	77 ± 2*	88 ± 4	83 ± 3
Reticular formation	72 ± 7	56 ± 1*	66 ± 2	58 ± 4
Inferior colliculus	178 ± 9	150 ± 17	188 ± 10	163 ± 9
Red n	79 ± 6	73 ± 2	75 ± 3	69 ± 5
Raphe nuclei				
Dorsal	84 ± 4	65 ± 3*	74 ± 6	56 ± 3#
Median	105 ± 5	86 ± 6**	100 ± 3	84 ± 5##
Super olive	124 ± 6	107 ± 7	110 ± 9	96 ± 8
Locus coeruleus	71 ± 4	63 ± 1	65 ± 6	69 ± 3
Inferior olive	70 ± 4	70 ± 2	72 ± 6	71 ± 4
Cerebellum				
Hemispheres	61 ± 3	47 ± 4*	48 ± 3	53 ± 4
Dentate n	109 ± 4	88 ± 7*	99 ± 5	89 ± 3
Interpositum n	98 ± 11	102 ± 3	99 ± 5	87 ± 5
Vermis	104 ± 8	90 ± 4	101 ± 5	94 ± 6

Data are mean ± SEM (μmol/100 g/minute) for groups of five to six animals. [^{14}C]DG was administered 120 minutes after CMI 10 mg/kg or saline. For each region rCMRglc values of columns 2 and 3 were compared to column 1: * $p < .05$, ** $p < .01$. Column 4 was compared to column 3: # $p < .05$, ## $p < .01$.

but minimally affected rCMRglc. Little or no long-term effects on resting rCMRglc have been observed after toxic (Freo et al. 1991; Pappius et al. 1988) or surgical manipulations of the 5-HT system (Cudennec et al. 1988) or after lesions of other brain structures such as locus coeruleus (Savaki et al. 1984), lateral habenula (Ito et al. 1985), or nucleus basalis of Meynert (Lamarca and Fibiger 1987; Soncrant et al. 1992). This may be due to compensatory mechanisms (e.g., receptor regulation, interneuronal circuitry) that restore resting rCMRglc (Soncrant et al. 1992). Alternatively, the 5-HT system may be tonically inactive, thereby contributing little to resting rCMRglc (Aghajanian 1972; Trulson et al. 1984).

In intact (saline-pretreated) animals, CMI (10 mg/kg) decreased rCMRglc in structures (e.g., raphe nuclei and superior colliculus) with high densities of 5-HT reuptake sites (De Souza and Kuyatt 1987) but not in structures with other receptor types [e.g., dopaminergic D_2 (Klemme et al. 1979), histaminergic H_1, adrenergic α_1 (Hornung et al. 1979), or serotoninergic 5-HT_2 receptors (McKenna and Peroutka 1989; Pazos et al. 1985)] that have been proposed as possible effectors of some CMI actions (Hall and Ogren 1981). In a previous study, higher doses (50 mg/kg) of CMI also decreased rCMRglc in areas with intermediate to low concentrations of 5-HT reuptake sites, suggesting that in individual brain areas CMI effects on rCMRglc depend on 5-HT reuptake site densities (Freo et al. 1993). Such a relation also was shown in the present study. PCA pretreatment abolished rCMRglc responses to CMI in most cortical and subcortical structures but not in the hippocampus and in the raphe nuclei, a finding that is consistent with the known differential vulnerability of 5-HT system components to PCA. Specifically, serotoninergic axons that originate from the dorsal raphe nucleus and innervate thalamic and basal ganglia structures appear more vulnerable to halogenated amphetamines than median raphe axons that innervate the hippocampus and lateral septum (Mamounas and Molliver 1988; Mamounas et al. 1991). Serotonergic cell bodies in both the dorsal raphe and median raphe nuclei appear morphologically unaffected by PCA (Bertilsson et al. 1975; Mamounas and Molliver 1988; Mamounas et al. 1991). Similarly, PCA decreases the binding of [^{3}H]-ligands (Dewar et al. 1992; Hensler et al. 1994) for 5-HT reuptake sites more severely in cortical areas than in the hippocampus and in the raphe nuclei, where rCMRglc responses to CMI were preserved. The current findings support the hypothesis that CMI reduces rCMRglc in telencephalic and diencephalic regions by interacting with 5-HT presynaptic elements, likely the 5-HT reuptake sites.

CMI is one the most potent 5-HT reuptake blockers (Kaspar et al. 1992), but it is not specific and, in rats, CMI increases synaptic concentrations of both 5-HT (Carboni and Di Chiara 1989) and norepinephrine (Kaspar et al. 1992; Kido et al. 1991). CMI (Feet and

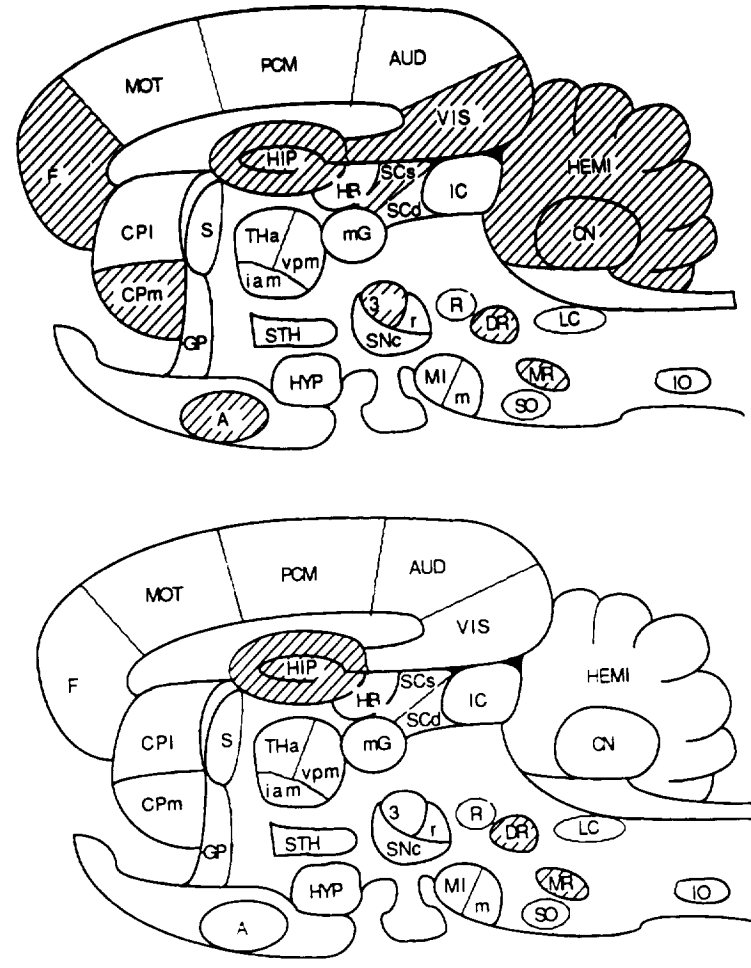

Figure 1. Schematic representation of changes in rCMRglc after CMI 10 mg/kg IP in rats pretreated with saline (*top*) or with PCA (*bottom*). Areas of rCMRglc decline compared to control are hatched. List of regions: *A*, amygdala; AUD, auditory cortex; CN, cerebellar nuclei; CPl, caudate-putamen, lateral part; CPm, caudate-putamen, medial part; DR, dorsal raphe; F, frontal cortex, area 10; GP, globus pallidus; HB, habenular complex, lateral nucleus; HEMI, cerebellar hemispheres; HIP, hippocampus; HYP, hypothalamus; iam, thalamus, interanteromedial nucleus; IC, inferior colliculus; IO, inferior olive; LC, locus coerulus; m, mammillary bodies, medial nucleus; mG, medial geniculate; MI, mammillary bodies, lateral nucleus; MOT, motor cortex; MR, median raphe; PCM, precentral medial cortex; r, substantia nigra, pars reticulata; R, red nucleus; S, medial septum nucleus; SCd, deep layer of the superior colliculus; SCs, superficial layer of superior colliculus; SNc, substantia nigra pars compacta; SO, superior olive; STH, subthalamic nucleus; THa, anterior thalamus; vpm, thalamus, ventroposteromedial nucleus; VIS, visual cortex; 3, oculomotor complex.

Gotestam 1994; Modigh et al. 1990), and other 5-HT reuptake blockers (e.g., fluoxetine, fluvoxamine) (Hoehn-Saric et al. 1993; Louie et al. 1993) are effective in treating panic attacks, a psychiatric disorder that has been linked most frequently to the noradrenergic system (Krystal et al. 1992; McDougle et al. 1994); intriguingly, 5-HT reuptake blockers also are effective in treating panic attacks presented by subjects who self-administered methylenedioxymethamphetamine ("Ecstasy") (Palanti and Mazzi, 1992; Whitaker-Azmitia and Aronson 1989) a psychotropic agent that induces a 5-HT axon degeneration indistinguishable from that caused by PCA (Mamounas and Molliver 1988; Mamounas et al. 1991; Ricaurte et al. 1986; Steele et al. 1994; Wilson et al. 1993). These findings suggest that CMI and other 5-HT reuptake blockers may act on the adrenergic system directly or via complex adrenergic-5-HT interactions (Feet and Gotestam, 1994; Hoehn-Saric et al. 1993; Kaplan 1992; Louie et al. 1993; Modigh et al. 1990). Unfortunately, human studies are limited because it is impossible to determine the nature and magnitude of the lesion(s) resulting in humans from amphetamine exposure. In the future, neuroimaging techniques such as position emission tomography may prove valuable in evaluating the status of 5-HT and norepinephrine reuptake sites in living patients if selective and specific ligands become available.

Theoretically, the noradrenergic system could contribute to the rCMRglc reductions induced by CMI in the raphe-hippocampal areas that were observed in PCA-lesioned rats. However, antidepressants with prevalent adrenergic actions, like CMI analogs and norepinephrine reuptake blockers (e.g., imipramine and desipramine) and MAO-A inhibitor (e.g., phenelzine), produce no rCMRglc change in raphe and hippocampal areas (Duncan et al. 1993; Gerber et al. 1983). In contrast, these brain regions, which are relatively spared by PCA (Bertilsson et al. 1975; Dewar et al. 1992; Hensler et al. 1994; Mamounas and Molliver 1988; Mamounas et al. 1991) and contain high brain concentrations of 5-HT reuptake sites, are sensitive to low doses of CMI (Freo et al. 1993). Only further lesion studies could positively rule out the involvement of the noradrenergic system in CMI actions, but the above findings suggest that 5-HT mechanisms mediate the CMI suppressant effects on rCMRglc in the raphe-hippocampal regions.

Reduced rCMRglc is associated with decreased electrical activity (Sokoloff et al. 1982), a reduction that can be produced in raphe neurons by both local and systemic administration of 5-HT reuptake blockers (Gal-

lager and Aghajanian 1975). Raphe areas appear more sensitive than cortical areas to neurochemical (Adell and Artigas 1991) and cerebral metabolic effects of CMI (Freo et al. 1993). The inhibitory effects of 5-HT reuptake blockers on raphe neurons may result from activation of 5-HT_{1A} somatodendritic autoreceptors by increased extracellular 5-HT (Adell and Artigas 1991). Hence, CMI therapeutic actions may depend on increased 5-HT receptor concentrations in 5-HT neuron somatic areas rather than in cortical projection areas.

In this study, we examined the effects of PCA-induced lesions on functional changes by acute administration of CMI. Further studies are needed to evaluate the effects of PCA pretreatment on chronic CMI administration that is necessary for the therapeutic effects in the treatment of depression, obsessive-compulsive disorders, and other psychiatric diseases.

In conclusion, the results indicate that (1) CMI decreases rCMRglc in areas with high densities of 5-HT uptake sites; (2) PCA pretreatment produces a severe decrease in 5-HT brain concentrations and in rCMRglc response to CMI and parallels the reported decrease in 5-HT reuptake sites; and (3) rCMRglc responses are unaffected in brain areas whose 5-HT reuptake sites are not decreased by PCA and these may be the primary clinically relevant targets of CMI.

REFERENCES

Adell A, Artigas F (1991): Differential effect of clomipramine given locally and systematically on extracellular 5-hydroxytryptamine in raphe nuclei and frontal cortex. Naunyn-Schmiederberg's Arch Pharmacol 343:237–244

Aghajanian GK (1972): Influence of drugs on the firing of serotonin-containing neurons in the brain. Fed Proc Fed Am Soc Exp Biol 31:91–96

Bertilsson L, Koslow SH, Costa E (1975): 5-Hydroxytryptamine depletion in mesencephalic nuclei of rat brain following single injection of p-chloroamphetamine. Brain Res 91:348–350

Carboni E, Di Chiara G (1989): Serotonin release estimated by transcortical dialysis in freely-moving rats. Neurosci 32:637–645

Carlsson A, Corrodi H, Fuxe K, Hokfelt T (1969): Effect of antidepressant drugs on the depletion of intraneural brain 5-hydroxytryptamine stores caused by 4-methyl-α-ethyl-meta-tyramine. Eur J Pharmacol 5:357–366

Cudennec A, Duverger D, Nishikawa T, McRae-Deguerce A, MacKenzie ET, Scatton B (1988): Influence of ascending serotonergic pathways on integrated functional activity in rat brain. I: Effects of electrolytic or neurotoxic lesions of the dorsal and/or median raphe nucleus. Brain Res 444:214–226

De Souza EB, Kuyatt BL (1987): Autoradiographic localization of ^{3}H-paroxetine-labeled serotonin uptake sites in rat brain. Synapse 1:488–496

Dewar KM, Grondin L, Carli M, Lima L, Reade TA (1992): [^{3}H]Paroxetine binding and serotonin content of rat cortical areas, hippocampus, neostriatum, ventral mesencephalic tegmentum and midbrain nuclei regions following p-chlorophenylalanine and p-chloroamphetamine treatment. J Neurochem 58:250–257

Duncan GE, Johnson KB, Breese GR (1993): Topographic patterns of brain activity in response to swim stress: Assessment by 2-deoxyglucose uptake and expression of Fos-like immunoreactivity. J Neurosci 13:3932–3943

Feet PO, Gotestam KG (1994): Increased antipanic efficacy in combined treatment with clomipramine and dioxyrazine. Acta Psychiatr Scand 89:230–234

Freo U, Larson DL, Tolliver T, Rapoport SI, Soncrant TT (1991): Parachloroamphetamine selectively alters regional cerebral metabolic responses to the serotonergic agonist meta-chloroamphetamine in rats. Brain Res 544:17–25

Freo U, Pietrini P, Dam M, Pizzolato G, Battistin L (1993): The tricyclic antidepressant clomipramine dose-dependently reduces regional cerebral metabolic rates for glucose in awake rats. Psychopharmacology 113:53–59

Fuller RW, Wong DT (1977): Inhibition of serotonin reuptake. Fed Proc 36:2154–2158

Gallager DW, Aghajanian GK (1975): Effects of chlorimipramine and lysergic acid diethylamide on efflux of precursor-formed ^{3}H-serotonin: Correlation with serotonergic impluse flow. J Pharmacol Exp Ther 193:785–793

Gerber JC III, Choki J, Brunswick DJ, Reivich M, Frazer A (1983): The effect of antidepressant drugs on regional cerebral glucose utilization in the rat. Brain Res 269:319–325

Hall H, Ogren SO (1981): Effects of antidepressant drugs on different receptors in the brain. Eur Pharmacol 70:393–407

Hensler JG, Ferry RC, Labow DM, Kovachich GB, Frazer A (1994): Quantitative autoradiography of the serotonin transporter to assess the distribution of serotoninergic projections from the dorsal raphe nucleus. Synapse 17:1–15

Hoehn-Saric R, McLeod DR, Hipsley PA (1993): Effect of fluvoxamine on panic disorder. J Clin Psychopharmacol 13:321–326

Hoffart A, Due-Madsen J, Lande B, Gude T, Bille H, Torgensen S (1993): Clomipramine in the treatment of agoraphobic in patients resistent to behavioral therapy. J Clin Psych 54:481–487

Hornung R, Preesek R, Glossmann H (1979): Alpha adrenoreceptors in rat brain: Direct identification with prazosin. Brain Res 323:211–222

Ito M, Kadekaro M, Sokoloff L (1985): Effects of lateral habenula lesions on local cerebral glucose utilization in the rat. Brain Res 337:245–255

Kaplan GB (1992): Neurobiological aspects of panic disorders. R I Med 75:233–235

Kaspar S, Fuger J, Moller H-J (1992): Comparitive efficacy of antidepressants. Drugs (suppl. 2):11–23

Kido H, Hasegawa M, Sakamoto H, Yamaguchi N, Kurata K, Kurachi M (1991): Effects of clomipramine on the concentrations of catecholamines, indoleamines and their metabolites in 11 rat brain regions. Jap J Psychiatry 45: 885–896

Klemme N, Murrin LC, Kuhar MJ (1979): Neuroleptic and dopamine receptors: Autoradiographic localization of [^{3}H]spiperone in rat brain. Brain Res 169:1–9

Krystal JH, McDougle CJ, Woods SW, Price LH, Heninger

GR, Charney DS (1992): Dose-response relationship for oral idazoxan effects in healthy human subjects: Comparison with oral yohimbine. Psychopharmacology 108: 313–319

Lamarca MV, Fibiger HC (1987): Deoxyglucose uptake and choline acetyltransferase activity in cerebral cortex following lesions of the nucleus basalis magnocellularis. Brain Res 307:366–369

Louie AK, Lewis TB, Lannon RA (1993): Use of low-dose fluoxetine in major depression and panic disorder. J Clin Psychiatry 54:435–438

Mamounas LA, Molliver M (1988): Evidence for dual serotonergic projections to neocrotex: Axons from the dorsal and median raphe nuclei are differentially vulnerable to the neurotoxin p-chloroamphetamine (PCA). Exp Neurol 102:23–26

Mamounas LA, Mullen CA, O'Hearn E, Molliver M (1991): Dual serotonergic projections to forebrain in the rat: Morphologically distinct 5-HT axon terminals exhibit differential vulnerability to neurotoxic amphetamine derivatives. J Comp Neurol 314:558–586

McDougle CJ, Black JE, Malison RT, Zimmerman RC, Kosten TR, Heinger GR, Price LH (1994): Noradrenergic dysregulation during discontinuation of cocaine use in addicts. Arch Gen Psychiatry 51:713–719

McKenna DJ, Peroutka SJ (1989): Differentiation of 5-hydroxytryptamine$_2$ receptor subtypes using ^{125}I-R-(−)2,5-domethoxy-4-iodo-phenylisopropylamine and [^{3}H]ketanserin. J Neurosci 9:3482–3490

Mefford IN (1981): Application of high performance liquid chromatography with electrochemical detection to neurochemical analysis: Measurements of cathecolamines, serotonin and metabolites in the brain. J Neurosci Meth 3:207–224

Miller RG (1966): Simultaneous Statistical Inference. New York, McGraw Hill

Modigh K, Westberg P, Eriksson E (1992): Superiority of clomipramine over imipramine in the treatment of panic disorder: A placebo-controlled trial. J Clin Psychopharmacol 12:251–261

Murphy DL (1990): Peripheral indices of central serotonin functions in humans. Ann N Y Acad Sci 600:282–295

Murphy DL, Pigott TA (1990): A comparative examination of a role for serotonin in obsessive compulsive disorder, panic disorder and anxiety. J Clin Psychiatry 51(suppl): 53–58

Pallanti S, Mazzi D (1992): MDMA (Ecstacy) precipitation of panic disorder. Biol Psychiatry 32:91–95

Pappius HM, Dadoun R, McHugh M (1988): The effect of p-chlorophenylalanine on cerebral metabolism and biogenic amine content of traumatized brain. J Cereb Blood Flow Metab 8:324–334

Paxinos G, Watson C (1982): The Rat Brain in Stereotactic Coordinates. New York, Academic Press

Pazos A, Cortes R, Palacios JM (1985): Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin-2 receptors. Brain Res 346:231–246

Peroutka SJ, Snyder SH (1980): Regulation of serotonin (5-HT) receptors labeled with [^{3}H]-spiperidol by chronic treatment with antidepressant. J Pharmacol Exp Ther 215: 582–587

Pizzolato G, Soncrant TT, Rapoport SI (1985): Time-course and regional distribution of the metabolic effect of bromocryptine in the rat brain. Brain Res 341:303–312

Ricaurte GA, Forno LS, Wilson MA (1986): 3,4-Methylenedioxymethamphetamine (MDMA): Stereoselective interaction at brain 5-HT$_1$ and 5-HT$_2$ receptors. Psychopharmacology 88:525–526

Savaki HE, Graham DI, Grome JJ, McCulloch J (1984): Functional consequences of unilateral lesion of the locus coreuleus: A quantitative [^{14}C]2-deoxyglucose investigation. Brain Res 292:239–249

Sokoloff L (1982): The radioactive deoxyglucose method—Theory, procedure and applications for the measurements of local glucose utilization in the central nervous system. Adv Neurochem 4:1–82

Sokoloff L, Reivich AA, Kennedy C, Des Rosiers MH, Patlak CS, Pettigrew KD, Sakurada O, Shinohara M (1977): The (^{14}C)2-deoxyglucose method for the measurement of local cerebral glucose metabolism. Theory, procedure and normal values in the conscious and anesthetized albino rat. J Neurochem 28:897–916

Soncrant TT, Holloway HW, Rapoport SI (1985): Arecoline-induced elevations of regional cerebral metabolism in the conscious rat. Brain Res 347:205–217

Soncrant TT, Holloway HW, Stipetic M, Rapoport SI (1988): Cerebral glucose utilization in rats is not altered by hindlimb restraint or by femoral artery and vein cannulation. J Cereb Blood Flow Metab 8:720–726

Soncrant TT, Holloway HW, Horwitz B, Rapoport SI, Lamour YA (1992): Effects of nucleus basalis magnocellularis ablation on local cerebral glucose utilization in the rat: Functional brain reorganization. Eur J Neurosci 4:653–662

Steele TD, McCann UD, Ricaurte GA (1994): 3,4-Methylenedioxymethamphetamine (MDMA, "Ecstasy"): Pharmacology and toxicology in animals and humans. Addiction 89:539–551

Sundblad C, Hedberg MA, Eriksson E (1993): Clomipramine administered during the luteal phase reduces the symptoms of premenstrual syndrome: A placebo-controlled trial. Neuropsychopharmacology 9:133–145

Thomas PC, Jones RB (1977): The effects of clomipramine and desmethylclomipramine on the in vitro uptake of radiolabelled 5-HT and noradrenaline into rat brain cortex slices. J Pharm Pharmacol 29:562–563

Trulson ME, Crisp T, Trulson VM (1984) Activity of serotonin-containing nucleus centralis superioris (raphe medianus) neurons in freely moving cats. Exp Brain Res 54:33–44

Whitaker-Azmitia PM, Aronson TA (1989): Ecstasy (MDMA) induced panic (letter). Am J Psychiatry 146:119

Wilson MA, Mamounas LA, Fasman KH, Axt KJ, Molliver ME (1993): Reactions of 5-HT neurons to drugs of abuse: Neurotoxicity and plasticity. In Erinoff L. (ed.), NIDA Research Monograph 136, Rockville, MD: U.S. Department of Health and Human Services, pp 155–178

Wozniak KM, Aulakh CS, Hill JL, Murphy DL (1989): Hyperthermia induced by MCPP in the rat and its modification by antidepressant treatment. Psychopharmacology 97: 269–274