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Local Cerebral Metabolic Effects of the Novel Cocaine Analog, WF-31: Comparisons to Fluoxetine

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ABSTRACT The effects of the acute administration of the selective serotonin uptake inhibitor, fluoxetine, on rates of local cerebral glucose utilization in rats were compared to those of the novel cocaine analog, [2β-propanoyl-3β-(4-isopropylphenyl)-tropane, WF-31, which has greater affinity for serotonin than dopamine transporters, using the quantitative autoradiographic 2-[^{14}C]deoxyglucose method. Locomotor activity was assessed simultaneously. Fluoxetine administration resulted in dose-dependent decreases in locomotor behavior, as well as widespread reductions in rates of metabolic activity in brain areas including raphe nuclei, dorsal and ventral striatum, amygdala, hippocampus, limbic cortex, and thalamus. These effects were largely concentrated in brain regions containing high densities of serotonin transporters as revealed by in vitro autoradiography. In contrast, the acute administration of WF-31 produced more discrete changes in metabolic activity that were localized within the raphe nuclei and in portions of the hippocampal formation. Blockade of WF-31's dopaminergic effects by pretreatment with the antagonist, α-flupenthixol, resulted in a pattern of metabolic changes that closely resembled that observed with fluoxetine. These data suggest that the alterations in functional activity produced by both fluoxetine and WF-31 are largely the result of actions on serotonergic systems. **Synapse 27:26–35, 1997.**

INTRODUCTION

One recent approach to improving the understanding of the effects of cocaine at monoamine transporters has been the use of derivatives of cocaine that vary in selectivity and potency from cocaine itself. Several groups have synthesized numerous novel tropane analogs that have contributed to our knowledge of the pharmacophore responsible for the actions of cocaine (Abraham et al., 1992; Boja et al., 1994; Carroll et al., 1992a,b, 1993; Davies et al., 1991, 1993, 1996; Kozikowski et al., 1991, 1992; Lewin et al., 1992). Since many of the behavioral effects of cocaine have been attributed to its actions at dopamine transporters (DeWit and Wise, 1977; Ritz et al., 1987), much of the effort has been extended toward the development of compounds that are highly potent and selective at dopamine transporters (Carroll et al., 1995; Kelkar et al., 1994; Meltzer et al., 1993). Studies of the effects of compounds such as these have demonstrated that these analogs have behavioral activating properties that are similar to those of cocaine and other dopamine uptake inhibitors and furthermore, parallel their potency at dopamine transporters in vitro (Cline et al., 1992; Porrino et al., 1994, 1995).

Cocaine, however, binds with equal affinity to both dopamine and serotonin transporters (Ritz et al., 1990), and recent studies have challenged the view that dopamine alone is responsible for the behavioral effects of cocaine and have emphasized the importance of serotonin function in mediating cocaine's effects. Manipulation of serotonergic systems can alter both cocaine-induced increases in locomotor activity (Cunningham et al., 1992; Pradhan et al., 1978; Reith et al., 1991) and self-administration behavior maintained by cocaine infusions (Carroll et al., 1990a; Loh and Roberts, 1990; Richardson and Roberts, 1991). Self-administration of cocaine, for example, is reduced by treatment with the serotonin uptake inhibitor, fluox-

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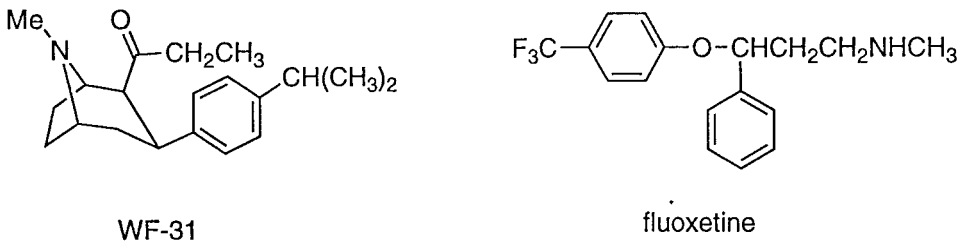

WF-31 fluoxetine

Fig. 1. Chemical structures of WF-31 and fluoxetine.

etine (Carroll et al., 1990a; Richardson and Roberts, 1991) or with the precursor 5-hydroxytryptophan (Carroll et al., 1990b), whereas the neurochemical destruction of forebrain serotonin increases the breakpoint for cocaine self-administration (Loh and Roberts, 1990), a measure considered a reflection of increased reinforcing effects of cocaine. It would appear then that increasing serotonergic tone inhibits cocaine-elicited effects, whereas destruction of serotonin potentiates the effects of cocaine. The neurobiological basis of these interactions, however, remains unclear. As has been seen with dopamine-selective analogs, the use of serotonin-selective agents may aid in elucidating aspects of the role of serotonin systems in the effects of cocaine, as well as increase our understanding of the role of serotonin systems in neuropsychiatric disorders, such as depression, obsessive-compulsive and eating disorders.

Davies and his colleagues have used a novel synthetic scheme based on vinylcarbenoid chemistry (Davies et al., 1991, 1994, 1996) to develop a series of cocaine analogs. This series contains compounds that have relatively high potencies at both dopamine and serotonin transporters (Bennett et al., 1995; Davies et al., 1994). In addition, this series also contains a tropane that has been shown to display a greater affinity for serotonin transporters that dopamine transporters, WF-31, [2β-propanoyl-3β-(4-isopropylphenyl)-tropane (Fig. 1). Both esters of the cocaine molecule have been removed in this compound and an isopropyl substituent placed on the 3-position of the tropane ring. In vitro assays showed that WF-31 is approximately 10 times more potent than cocaine at binding to serotonin transporters and at inhibiting serotonin uptake with K_i values of 49 and 36 nM in these assays, respectively (Bennett et al., 1995; Davies et al., 1993, 1994). Furthermore, besides being more potent, WF-31 is relatively selective for serotonin transporters, as it is approximately 12–15 times more potent at serotonin than dopamine transporters (Bennett et al., 1995). Although other groups have developed similar compounds (Blough et al., 1996), no data are yet available on their actions in vivo. Despite only relative selectivity for serotonin sites, the availability of WF-31 has allowed the present studies to evaluate for the first time whether a serotonin-selective tropane had cerebral metabolic effects similar to fluoxetine, a highly selective serotonin reuptake inhibitor (Fuller et al., 1974; Fig. 1).

Metabolic mapping with quantitative autoradiographic 2-[^{14}C]deoxyglucose method (2-DG) for the measurement of rates of glucose utilization (Sokoloff et al., 1977) has been used frequently to determine the central effects of cocaine and related tropane analogs (London et al., 1986; Porrino, 1993; Porrino et al., 1988, 1995). Because of the close coupling between energy metabolism and functional activity in the central nervous system, this approach provides a means to identify those brain regions in which functional activity is altered by the administration of a drug (Sokoloff and Porrino, 1986). The purpose of the present study, therefore, was to identify the neuroanatomical substrates of the effects of the acute administration of the cocaine analog, WF-31. Furthermore, these effects were compared to those that result from the administration of the nontropane, serotonin selective reuptake inhibitor, fluoxetine, in order to determine whether WF-31's functional effects can be attributed to its actions at serotonin transport sites.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 300–325 g at the time of the experiment were used in all studies. Animals were housed two per cage in standard hanging rodent cages with food and water available ad libitum under standard controlled lighting (12-hour light-dark cycle; lights on 05:00–17:00 h) and temperature conditions. All procedures were carried out in accordance with established practices as described in the NIH guide for care and use of laboratory animals. In addition, all procedures were reviewed and approved by the Animal Care and Use Committee of Bowman Gray School of Medicine of the Wake Forest University.

On the day prior to the measurement of rates of local cerebral glucose metabolism, rats were anesthetized with a mixture of halothane and nitrous oxide. Polyethylene catheters were inserted into a femoral vein and artery and run subcutaneously exiting at the nape of the neck. Such catheter placement allows the intravenous administration of drug or tracer and permits animals to move freely throughout the experimental procedure (Crane and Porrino, 1989).

Drugs

WF-31 was synthesized according to the procedures described by Davies et al. (1991, 1994) and was dissolved in buffered saline. Doses of WF-31 were based on the results of in vitro uptake studies in which WF-31 was shown to have a serotonin:dopamine uptake ratio of 0.069. Fluoxetine (Lilly, Indianapolis, IN) was dissolved in buffered saline. α-Flupenthixol was purchased from Research Biochemicals International, Inc. (Natick, MA) and was dissolved in 50 mM Tris buffer. The dose of α-flupenthixol, 0.1 mg/kg, was chosen based on previous experiments in our laboratory (Williams-Hemby and Porrino, submitted; Daunais et al., submitted). All drug and vehicle solutions were prepared fresh on the day of the experiments.

Procedures

In the first set of experiments, the effects of the acute administration of WF-31 on spontaneous locomotor activity and rates of cerebral glucose utilization were compared to the effects of the acute administration of fluoxetine. Locomotor activity was measured simultaneous to the measurement of rates of glucose utilization. Locomotor activity was measured in open-field Plexiglas test chambers ($42 \times 42 \times 30$ cm) by electronic counters that detected interruptions of eight independent photocell beams (Omnitech, Columbus, OH). Photocell counts were recorded and stored at 5-min intervals. Rats were habituated to locomotor test chambers for two consecutive days for 60 min each day prior to testing. On the day of the experiment, rats were placed in the test chambers 20 min before intraperitoneal injection of WF-31 (0.0, 1.0, or 10 mg/kg; $n = 4$, 5, and 5, respectively) or fluoxetine (0.0, 3.0, 10.0, 17.0 mg/kg; $n = 4$ each group). Arterial and venous catheters were extended out the top of the Plexiglass box, while rats remained in the chamber. The measurement of local rates of cerebral glucose utilization was initiated 30 min postinjection by the infusion of pulse of radioactive tracer through the femoral venous catheter as described below.

Because WF-31 displays affinity for the dopamine transporter as well as the serotonin transporter, the potential contribution of dopaminergic systems to the effects of the WF-31 on rates of local cerebral glucose utilization was evaluated by pretreatment with the dopaminergic antagonist, α-flupenthixol. α-Flupenthixol (0.1 mg/kg) was administered 1.5 h prior to WF-31 (10 mg/kg) and the procedure for the measurement of rates of glucose utilization initiated 30 minutes later.

Measurement of rates of local cerebral glucose utilization

Local rates of glucose utilization were measured according to the procedures described by Sokoloff et al. (1977) as adapted for use in freely moving animals (Crane and Porrino, 1989). The procedure was initiated by the infusion of an intravenous pulse of 2-deoxy-D-[1-^{14}C]glucose at the dose of 100 μCi/kg (New England Nuclear, Boston, MA; specific activity 50–55 mCi/mmol) followed by a flush of heparinized saline. Timed arterial blood samples were drawn thereafter at a schedule sufficient to define the time course of the concentrations of arterial 2-[^{14}C]DG and plasma glucose. Arterial blood samples were centrifuged immediately. Plasma concentrations of 2-[^{14}C]DG were determined by liquid scintillation spectrophotometry (Beckman Instruments, Fullerton, CA) and plasma glucose concentrations assessed with a glucose analyzer (Beckman Instruments). Approximately 45 min after tracer injection, the animals were killed by an intravenous overdose of sodium pentobarbital (100 mg/kg, iv). Brains were rapidly removed, frozen in isopentane (−45°C) and stored at −70°C. Coronal sections (20-μm-thick) were cut in a cryostat maintained at −22°C. Five of every ten sections were thaw-mounted on glass coverslips, dried on a plate, and autoradiographed with Kodak EMC or MIN-R X-ray film, along with a set of [^{14}C]methylmethacrylate standards (Amersham, Arlington Heights, IL), previously calibrated for their equivalent ^{14}C concentration in 20-μm brain sections.

Autoradiograms were analyzed by quantitative densitometry with a computerized image-processing system (MCID, Imaging Research, St. Catharines, Ontario). Optical density measurements for each structure, identified by direct comparison with adjacent or nearly adjacent sections stained with thionin, were made in a minimum of five brain sections. Tissue ^{14}C concentrations were determined from the optical densities and a calibration curve obtained by densitometric analysis of the autoradiograms of the calibrated standards. Rates of glucose utilization was then calculated using the local ^{14}C tissue concentrations, the time courses of the plasma glucose and ^{14}C concentrations and the appropriate constants according to the operational equation of the method (Sokoloff et al., 1977).

Statistical analysis

Behavioral data were analyzed by means of a Dunnett's t-test for multiple comparisons following a one-way analysis of variance. Rates of local cerebral glucose utilization were determined in 38 discrete brain structures. Statistical analysis was carried out on each region individually by means of Dunnett's t-tests for multiple comparisons following a one-way analysis of variance. The effects of the administration of fluoxetine and WF-31 were analyzed separately, although vehicle control groups were combined, as no differences were obtained between them on any measure. Finally, in order to determine the contribution of dopaminergic systems to the cerebral metabolic effects of WF-31, rates of cerebral glucose utilization in rats treated with

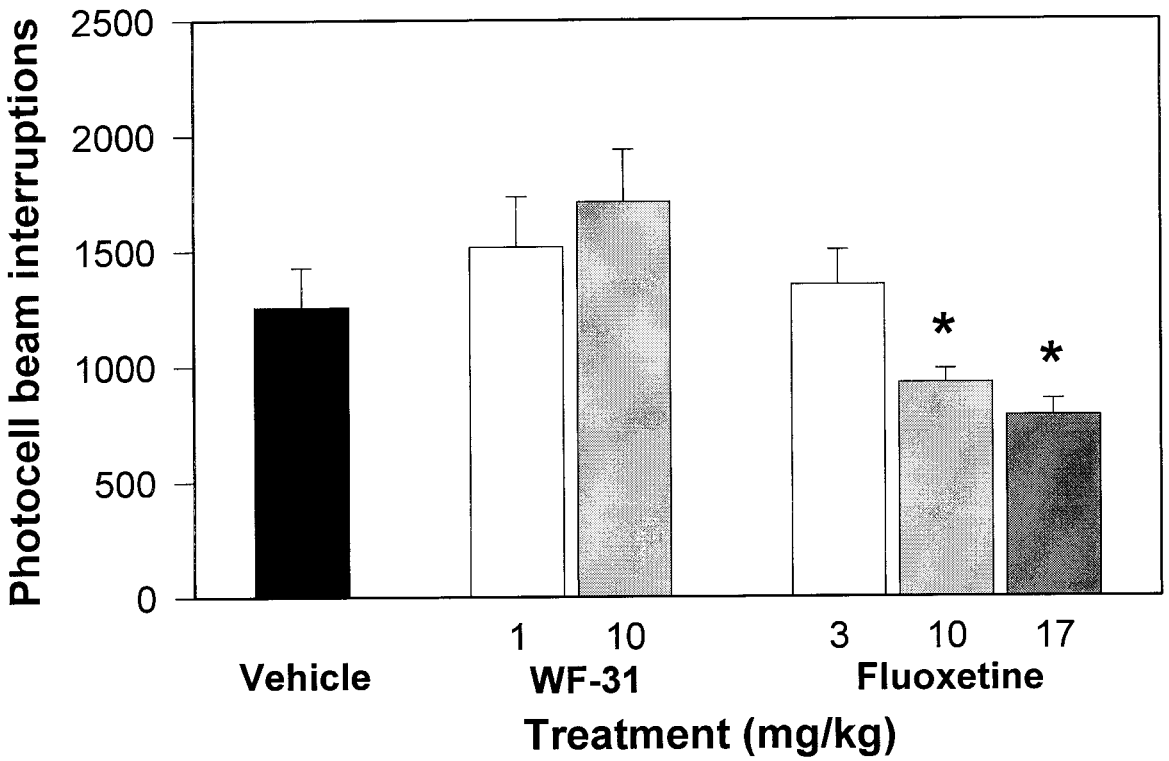

Fig. 2. Effects of the acute administration of WF-31 and fluoxetine on horizontal or total activity measured during the 45-min 2-[^{14}C]deoxyglucose procedure. Data shown are mean ± S.E.M. photocell counts for n = 8 vehicle, n = 4, 5 WF-31, 1.0, and 10.0 mg/kg, respectively, and n = 5, 4, 4 fluoxetine, 3.0, 10.0, and 17.0 mg/kg, respectively. Data were analyzed with Dunnett's tests comparing each dose of each drug with vehicle controls (* = $P < .05$, different from vehicle controls).

WF-31 (10 mg/kg) were compared to those in rats treated with α-flupenthixol (0.1 mg/kg) prior to WF-31 treatment by means of Student t-tests.

RESULTS

Behavioral effects

Locomotor activity was measured during the 45-min experimental period during which rates of glucose utilization were assessed simultaneously. The effects of the administration of WF-31 and fluoxetine in this context are shown in Figure 2. WF-31 did not significantly alter spontaneous locomotor activity, as compared to levels of activity elicited by vehicle treatment. There was, however, a trend toward increased behavioral activity particularly at the higher dose tested, 10 mg/kg. This was most evident when levels of horizontal activity were compared. In contrast, fluoxetine administration reduced levels of spontaneous activity dose-dependently as compared to vehicle-treated controls, with significant differences noted at the higher doses tested, 10.0 and 17.0 mg/kg. Similar effects were evident when levels of forward locomotion and stereotypic behavior were examined (data not shown).

Rates of local cerebral glucose utilization

The acute administration of fluoxetine produced dose-dependent reductions in rates of cerebral metabolism throughout the brain and are shown in Table I. At the lowest dose tested, 3 mg/kg, fluoxetine administration did not significantly change rates of glucose utilization in any brain region examined. The administration of moderate doses of fluoxetine, 10 mg/kg, however, produced widespread decreases in glucose utilization in 10 of the 37 brain areas including the dorsal (−23%) and median (−23%) raphe nuclei, as well as dorsolateral (−14%) and ventral (−13%) portions of the caudate nucleus. Metabolic activity was also decreased in portions of the mesocorticolimbic system, specifically in the nucleus accumbens (−12–13%), the medial prefrontal cortex (−12%), anterior cingulate cortex (−15%), olfactory tubercle (−14%) and ventral tegmental area (−16%), as well as in portions of the hippocampal formation (−11–13%). The administration of the highest dose of fluoxetine, 17.0 mg/kg, produced large, widespread significant reductions in cerebral metabolism ranging from 15% to 30% in 23 of the 37 structures examined. In addition to the areas affected by the moderate dose, glucose utilization was also depressed in septum, dorsal and ventral pallidum, basolateral amygdala, entopeduncular nucleus, substantia nigra reticulata, medial thalamus, and locus coeruleus (Table I; Fig. 3).

In contrast, the acute administration of WF-31 resulted in discrete, highly selective alterations in rates

TABLE I. Effects of the acute administration of fluoxetine on rates of local cerebral glucose utilization (μmol/100 g/min)

Structure	Vehicle	Fluoxetine		
	0.0 mg/kg (n = 8)	3.0 mg/kg (n = 5)	10.0 mg/kg (n = 4)	17.0 mg/kg (n = 4)
	mean ± S.E.M.			
Mesocorticolimbic system				
Medial prefrontal cortex	79.2 ± 1.3	80.9 ± 5.5	69.7 ± 2.1*	60.8 ± 2.2*
Anterior nucleus accumbens	100.2 ± 2.9	107.9 ± 5.3	87.6 ± 1.5*	81.6 ± 4.6*
Central nucleus accumbens	103.3 ± 2.4	101.1 ± 4.8	88.8 ± 2.3*	79.2 ± 4.8*
Posterior nucleus accumbens	88.1 ± 2.6	81.1 ± 4.9	75.5 ± 3.3	65.1 ± 4.8*
Shell of the nucleus accumbens	90.3 ± 3.9	81.4 ± 5.8	78.7 ± 5.4	67.1 ± 5.4*
Anterior olfactory tubercle	109.7 ± 3.6	108.6 ± 4.2	94.1 ± 1.3*	82.9 ± 4.2*
Central olfactory tubercle	105.2 ± 3.7	104.3 ± 4.1	92.7 ± 0.7	79.2 ± 5.4*
Posterior olfactory tubercle	85.2 ± 3.8	75.8 ± 3.2	72.8 ± 5.1	60.8 ± 6.6*
Anterior cingulate cortex	111.9 ± 2.6	121.4 ± 5.1	94.8 ± 2.8*	85.4 ± 6.5*
Ventral pallidum	63.7 ± 0.6	62.7 ± 3.2	58.7 ± 1.1	49.2 ± 3.4*
Lateral septum	63.7 ± 0.9	66.4 ± 3.6	57.6 ± 1.4	52.3 ± 4.0*
Basolateral amygdala	86.2 ± 3.4	89.5 ± 6.9	75.3 ± 2.7	67.9 ± 9.5*
Central amygdala	52.2 ± 1.6	50.7 ± 3.6	49.4 ± 1.5	42.4 ± 3.7
Medial forebrain bundle	70.8 ± 2.3	70.5 ± 4.0	60.7 ± 1.7	55.3 ± 7.0*
Ventral tegmental area	69.0 ± 2.2	74.9 ± 3.4	57.7 ± 0.8*	58.3 ± 4.8*
Nigrostriatal system and related areas				
Caudate (dorsomedial)	109.8 ± 4.0	120.8 ± 6.4	94.0 ± 2.9	85.1 ± 4.0*
Caudate (dorsolateral)	112.9 ± 3.2	124.6 ± 6.9	97.3 ± 2.5*	90.7 ± 5.6*
Caudate (ventral)	107.2 ± 3.0	112.0 ± 5.6	93.1 ± 1.7*	85.6 ± 5.3*
Globus pallidus	60.0 ± 1.1	61.3 ± 3.8	54.4 ± 0.6	45.6 ± 3.3*
Entopeduncular nucleus	60.2 ± 1.6	60.6 ± 4.5	52.1 ± 2.8	47.9 ± 5.1*
Medial habenula	79.4 ± 2.4	86.2 ± 3.7	71.7 ± 2.3	69.4 ± 6.6
Lateral habenula (medial)	99.9 ± 5.2	110.4 ± 4.3	85.8 ± 4.6	84.5 ± 11.4
Lateral habenula (lateral)	108.0 ± 4.7	120.4 ± 5.7	93.0 ± 3.3	96.9 ± 13.5
Subthalamic nucleus	97.4 ± 3.3	101.2 ± 4.5	85.4 ± 3.3	89.2 ± 9.4
Substantia nigra compacta	76.5 ± 3.1	82.0 ± 5.0	65.6 ± 1.3	63.9 ± 6.9
Substantia nigra reticulata	61.7 ± 1.7	63.4 ± 3.5	55.5 ± 1.9	52.4 ± 5.4*
Other forebrain areas				
Motor cortex (FR1)	103.1 ± 2.0	107.4 ± 6.8	93.4 ± 1.7	89.9 ± 5.9
Corpus callosum	42.1 ± 0.6	42.4 ± 3.6	39.5 ± 0.7	34.4 ± 2.8
Lateral thalamus	90.0 ± 2.3	98.3 ± 6.4	76.9 ± 2.3	84.3 ± 7.3
Mediodorsal thalamus	106.0 ± 4.2	118.5 ± 7.3	90.4 ± 1.9	92.1 ± 10.2*
Hippocampus (CA1)	63.6 ± 3.2	69.2 ± 4.1	56.8 ± 0.9	52.9 ± 6.5
Hippocampus (CA3)	76.8 ± 3.9	81.7 ± 4.4	66.5 ± 0.2	62.4 ± 7.6
Hippocampus (dentate)	59.7 ± 1.1	60.1 ± 4.3	53.8 ± 1.6	53.2 ± 2.7
Other midbrain and brainstem areas				
Dorsal raphe	103.4 ± 1.8	97.4 ± 6.1	79.1 ± 2.9*	75.4 ± 4.6*
Median raphe	112.4 ± 4.6	108.5 ± 7.2	86.9 ± 4.1*	78.2 ± 5.0*
Locus coeruleus	64.8 ± 1.0	63.7 ± 4.2	53.8 ± 3.5*	48.3 ± 3.6*
Cerebellar cortex	65.0 ± 1.3	61.5 ± 4.4	57.0 ± 3.2	55.3 ± 2.2

*$P < .05$, different from 0.0 mg/kg, Newman-Keuls test.

of local cerebral glucose utilization. Rates of glucose utilization in the 37 structures examined are shown in Table II. The administration of the lower dose of WF-31 produced highly circumscribed changes in metabolism in that its administration significantly reduced cerebral metabolism only in the median raphe nucleus (−11%), but did not alter metabolism in any other brain region assessed. The higher dose of WF-31, 10 mg/kg, produced more widespread alterations, decreasing metabolic activity in both the median (−16%) and dorsal (−17%) raphe nuclei, as well as in the CA1 and CA3 subfields, and dentate gyrus of the hippocampal formation (Fig. 3). Rates of glucose utilization were unaltered in other brain regions.

In order to determine the contribution of the actions of WF-31 at dopamine transporters to its effects on cerebral metabolism, dopaminergic activity was inhibited by pretreatment with the dopaminergic antagonist, α-flupenthixol. These data are also shown in Table II. Rates of glucose utilization were significantly reduced in the dorsal and median raphe as well as in the hippocampus in rats pretreated with α-flupenthixol prior to WF-31 administration when compared to vehicle-treated controls. In other words, α-flupenthixol pretreatment failed to alter the effects of WF-31 treatment in these structures. Pretreatment with α-flupenthixol prior to WF-31, however, resulted in significant alterations in cerebral metabolism in portions of the mesocorticolimbic system, including the nucleus accumbens, olfactory tubercle, ventral pallidum, lateral septum, and the ventral tegmental area as compared to groups treated with either vehicle or WF-31 alone. Changes in metabolism within these structures were not evident following the administration of WF-31 alone. In addition, rates of glucose utilization in portions of the nigrostriatal system were also reduced by pretreatment with α-flupenthixol prior to the administration of WF-31 as compared to vehicle-treated con-

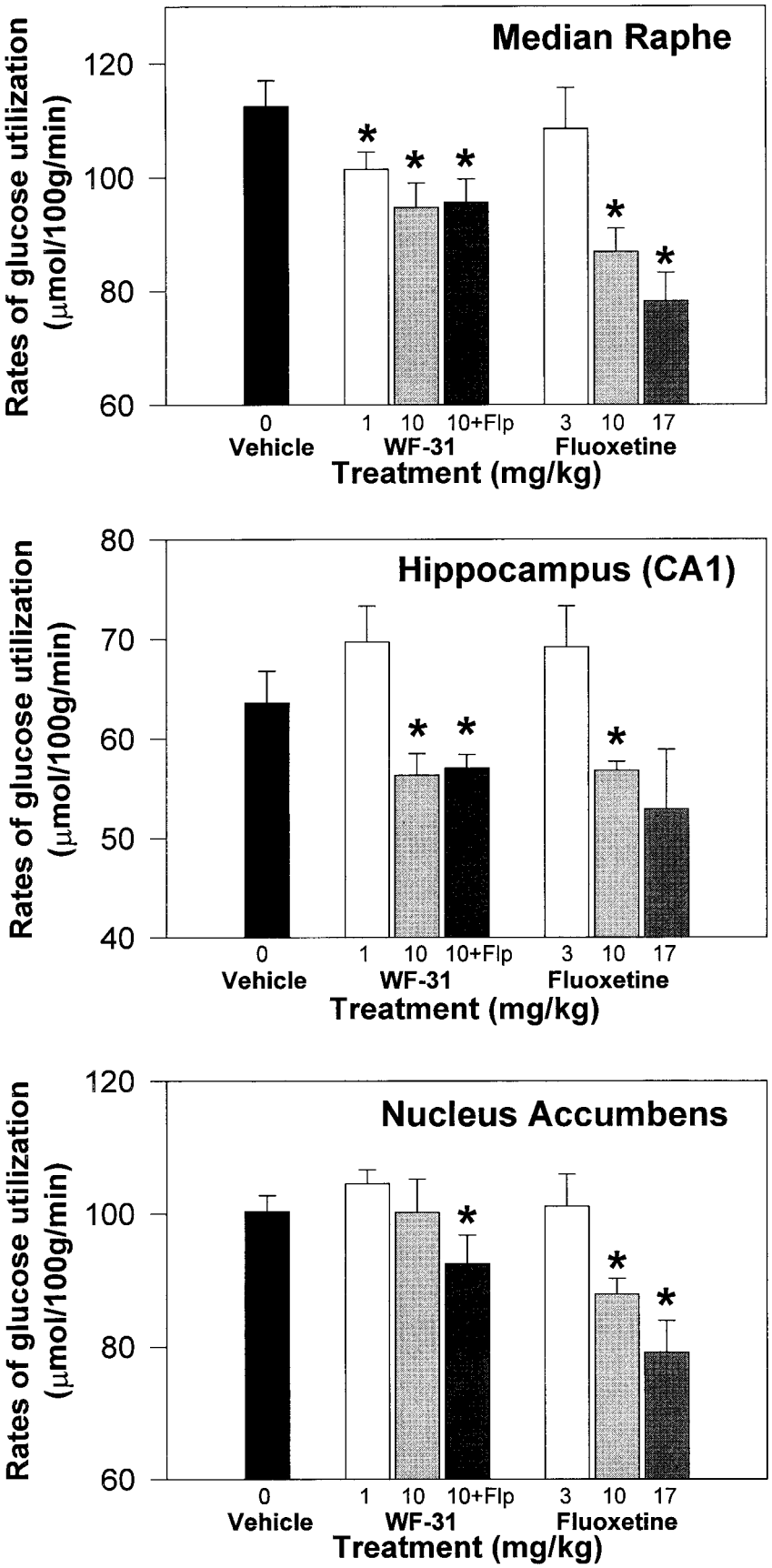

Fig. 3. Effects of the acute administration of WF-31, WF-31 with α-flupenthixol pretreatment (10+), and fluoxetine on rates of local cerebral glucose utilization in the median raphe nucleus (**upper**), CA1 subfield of the hippocampus (**center**), and the central portion of the nucleus accumbens (**lower**). Data shown are mean ± S.E.M. μmol/100 g/min for n = 8 vehicle; n = 4, 5 WF-31, 1.0, and 10.0 mg/kg, respectively; n = 5 WF-31, 10 mg/kg + α-flupenthixol; and n = 5, 4, 4 fluoxetine, 3.0, 10.0, and 17.0 mg/kg, respectively. Data were analyzed with Dunnett's tests comparing each dose of each drug with vehicle controls (* = $P < .05$, different from vehicle controls).

TABLE II. Effects of the acute administration of WF-31 on rates of local cerebral glucose utilization (μmol/100 g/min)

Structure	Vehicle	WF-31		
	0.0 mg/kg (n = 8)	1.0 mg/kg (n = 4)	10.0 mg/kg (n = 5)	Flupenthixol + 10.0 mg/kg (n = 5)
	mean ± S.E.M.			
Mesocorticolimbic system				
Medial prefrontal cortex	79.2 ± 1.3	81.2 ± 1.4	78.8 ± 2.8	79.7 ± 3.2
Anterior nucleus accumbens	100.2 ± 2.9	103.2 ± 5.0	99.9 ± 3.1	109.0 ± 3.0**
Central nucleus accumbens	103.3 ± 2.4	104.5 ± 2.1	100.2 ± 5.7	94.5 ± 4.3
Posterior nucleus accumbens	88.1 ± 2.6	88.0 ± 2.6	85.3 ± 5.3	71.6 ± 4.1*,**
Shell of the nucleus accumbens	90.3 ± 3.9	91.2 ± 4.5	86.7 ± 6.2	73.5 ± 3.5*
Anterior olfactory tubercle	109.7 ± 3.6	113.4 ± 3.4	111.1 ± 6.9	113.4 ± 5.7
Central olfactory tubercle	105.2 ± 3.7	106.6 ± 2.3	102.1 ± 6.1	98.8 ± 5.1
Posterior olfactory tubercle	85.2 ± 3.8	87.2 ± 4.7	77.2 ± 4.4	69.6 ± 4.3*
Anterior cingulate cortex	111.9 ± 2.6	119.9 ± 7.0	113.5 ± 7.0	95.0 ± 3.6
Ventral pallidum	63.7 ± 0.6	66.3 ± 2.0	60.1 ± 2.5	43.8 ± 2.3*,**
Lateral septum	63.7 ± 0.9	65.0 ± 3.6	61.3 ± 2.9	50.0 ± 3.6*,*
Basolateral amygdala	86.2 ± 3.4	90.7 ± 3.1	84.2 ± 4.0	88.7 ± 2.8
Central amygdala	52.2 ± 1.6	49.7 ± 1.3	51.0 ± 1.9	57.2 ± 1.4
Medial forebrain bundle	70.8 ± 2.3	71.7 ± 2.7	67.9 ± 4.3	70.6 ± 2.5
Ventral tegmental area	69.0 ± 2.2	73.4 ± 2.5	70.6 ± 2.7	58.8 ± 2.8*,**
Nigrostriatal system and related areas				
Caudate (dorsomedial)	109.8 ± 4.0	119.8 ± 6.3	108.1 ± 8.6	87.6 ± 5.9*
Caudate (dorsolateral)	112.9 ± 3.2	125.1 ± 8.5	111.9 ± 7.7	95.5 ± 7.2*
Caudate (ventral)	107.2 ± 3.0	114.6 ± 5.7	105.6 ± 6.0	82.6 ± 5.1*,**
Globus pallidus	60.0 ± 1.1	63.4 ± 2.3	55.6 ± 3.4	37.6 ± 3.0*,**
Entopeduncular nucleus	60.2 ± 1.6	61.1 ± 3.3	56.5 ± 3.3	61.1 ± 2.8
Medial habenula	79.4 ± 2.4	83.9 ± 3.3	76.6 ± 3.9	77.5 ± 2.3
Lateral habenula (medial)	99.9 ± 5.2	107.9 ± 3.2	95.5 ± 6.1	105.5 ± 5.2
Lateral habenula (lateral)	108.0 ± 4.7	119.0 ± 4.3	106.3 ± 7.1	134.1 ± 10.0*
Subthalamic nucleus	97.4 ± 3.3	105.8 ± 3.8	97.0 ± 4.8	77.3 ± 4.6*
Substantia nigra compacta	76.5 ± 3.1	82.3 ± 2.4	76.8 ± 3.6	56.0 ± 2.8*,**
Substantia nigra reticulata	61.7 ± 1.7	66.1 ± 2.4	61.2 ± 2.1	42.2 ± 2.6*,**
Other forebrain areas				
Motor cortex (FR1)	103.1 ± 2.0	111.9 ± 3.0	107.1 ± 5.1	96.0 ± 7.2
Corpus callosum	42.1 ± 0.6	43.6 ± 3.2	40.4 ± 1.3	32.1 ± 2.3*
Lateral thalamus	90.0 ± 2.3	96.2 ± 3.0	92.0 ± 6.2	97.6 ± 6.0
Mediodorsal thalamus	106.0 ± 4.2	113.4 ± 5.4	109.0 ± 7.8	108.6 ± 4.9
Hippocampus (CA1)	63.6 ± 3.2	69.7 ± 3.6	56.3 ± 2.2*	60.6 ± 3.2
Hippocampus (CA3)	76.8 ± 3.9	83.3 ± 3.0	64.8 ± 1.4*	70.6 ± 2.4*
Hippocampus (dentate)	59.7 ± 1.1	60.4 ± 3.3	54.1 ± 2.1*	57.1 ± 3.2
Other midbrain and brainstem areas				
Dorsal raphe	103.4 ± 1.8	97.6 ± 1.4	85.5 ± 4.2*	88.5 ± 4.5*
Median raphe	112.4 ± 4.6	101.4 ± 3.1*	94.7 ± 4.3*	96.6 ± 3.9*
Locus coeruleus	64.8 ± 1.0	64.1 ± 4.2	59.1 ± 2.6	55.6 ± 2.2
Cerebellar cortex	65.0 ± 1.3	65.1 ± 2.6	61.9 ± 2.3	52.6 ± 2.9

*$P < .05$, different from 0.0 mg/kg, Newman-Keuls test.
**$P < .05$ different from 10 mg/kg, Student's t-test.

trols. These structures included the caudate nucleus, globus pallidus, and substantia nigra compacta and reticulata. α-Flupenthixol pretreatment, then, failed to alter WF-31-induced reductions in rates of glucose utilization in the raphe and hippocampal formation, but decreased metabolism in dopaminergically innervated areas of the mesocorticolimbic and nigrostriatal systems.

DISCUSSION

The present results are the first report of the dose-dependent effects of the acute administration of the serotonin-selective reuptake inhibitor, fluoxetine, on rates of local cerebral glucose utilization. In these experiments, fluoxetine administration produced widespread dose-dependent decreases in metabolic activity in the raphe nuclei, amygdala, dorsal and ventral striatum, basal ganglia, hippocampus, and limbic cortical areas. There are strong similarities between this pattern of alteration in metabolic activity and that obtained following the administration of other serotonin agonist agents (cf. Freo et al., 1993a; Freo, 1996; Grome and Harper, 1986). Treatment with the serotonin-$5HT_2$ agonist, m-CPP, at doses that reduced locomotor activity, for example, also depressed functional activity in basal ganglia, dorsal and ventral striatum, raphe nuclei, amygdala, and cortical regions (Freo et al., 1992), as did the administration of quipazine (Freo et al., 1993b). The topography of these changes is clearly consistent with the changes induced by fluoxetine in the present study. Furthermore, the administration of a range of doses of clomipramine, a tricyclic antidepressant with affinity for serotonin transporters (Freo et al., 1993a), and a high dose of fluoxetine in rats (Merico et al., 1995) and humans (Cook et al., 1994), also produced similar decreases in cortex, striatum and brainstem.

The metabolic effects in these studies and those in the present study were largely localized in brain areas with high densities of serotonin transporters (Hrdina et al., 1990; Plenge et al., 1990). The largest decreases in metabolism following fluoxetine administration in the present study were found in the median and dorsal raphe nuclei, regions that have the highest concentration of serotonin transporters in brain. High densities of transporters are also present in amygdala, basal ganglia and striatum (Hrdina et al., 1990; Plenge et al., 1990), all of which displayed altered rates of functional activity in these studies.

The close similarity of the profile of changes of cerebral metabolism observed following the acute administration of the novel tropane analog, WF-31, to those of fluoxetine in the present study as well as other serotonin reuptake inhibitors and receptor agonists, strongly suggests that the changes that accompany WF-31 administration are the result of the actions of this compound on serotonin systems. This finding is consistent with the profile of WF-31 in in vitro assays, in which WF-31 has been shown to display a higher affinity for serotonin transporters than for dopamine and norepinephrine transporter sites. Further support for the serotonergic nature of WF-31's actions derives from the differences in the pattern of metabolic changes observed following its administration and those obtained following the administration of direct and indirect dopaminergic agonists (McCulloch et al., 1982; Orzi et al., 1983; Porrino et al., 1988, 1995; Porrino and Lucignani, 1987). Dopaminergic agonists generally produce widespread elevations in metabolic rates in conjunction with intense increases in behavioral activity.

Direct comparisons of the effects of fluoxetine and WF-31 are somewhat difficult, however, because the effects of each drug on functional activity are the result of the interaction of a number of dynamic processes including rate of absorption, duration of action, rate of metabolism, etc. Since both fluoxetine and WF-31 have relatively long durations of action, for example, study of their actions at time points other than those chosen in the present study may yield distinctly different patterns of changes in glucose utilization. Nonetheless, the similarities between the metabolic effects of fluoxetine and WF-31 strongly suggest that their effects are the result of their actions at serotonin transporter sites.

The correspondence in the distribution of the patterns of changes accompanying the administration of fluoxetine and WF-31 on cerebral metabolic activity is also consistent with the similarity of the effects of these two drugs in the forced swim test (Hemby et al., in press). In this assay, which serves as a measure of potential antidepressant effects (Porsolt et al., 1978, 1979), selective serotonin uptake inhibitors such as fluoxetine, sertraline and paroxetine significantly reduce immobility in a manner similar to tricyclic antidepressants and monoamine oxidase inhibitors (Detke et al., 1995). These effects are in contrast to those of dopaminergic uptake inhibitors which, although increase swimming like WF-31, also induce significant increases in general activity, not observed following fluoxetine or WF-31 treatment (Hemby et al., in press). WF-31, therefore, displays a spectrum of action in vivo consistent with its in vitro profile as a serotonin selective reuptake inhibitor.

The absence of more widespread changes in cerebral metabolism following the administration of WF-31, as compared to the traditional serotonin selective reuptake inhibitor, fluoxetine, may have been the result of the failure to test sufficiently high doses of WF-31, especially in view of the fact that fluoxetine is approximately six times more potent at serotonin transporters that WF-31. In in vivo assays, however, when equivalent doses of fluoxetine and WF-31 ranging from 0.3 to 10.0 mg/kg are tested, WF-31 is more effective than fluoxetine, for example, in decreasing immobility and increasing swimming in the forced swim test. Another possible explanation for the discrepancies between the effects of these two compounds that should be considered is the action of WF-31 at dopamine transporters in addition to its actions at serotonin sites. Dopamine uptake inhibitors including PTT, a tropane analog synthesized with the same strategy as WF-31, produce large increases in metabolic rates in striatal and extrastriatal mesocorticolimbic brain regions (Porrino et al., 1995). Although WF-31 is more potent at serotonin than at dopamine sites (Bennett, et al., 1995), its actions at dopamine transporters may counteract the depressant effects on metabolism that result from serotonin uptake inhibition. To address this possibility, rats were pretreated with the dopaminergic antagonist, α-flupenthixol, prior to the administration of WF-31 in order to block the effects due to dopaminergic stimulation. Inhibition of dopaminergic activity resulted in a pattern of glucose utilization alterations that more closely approximated that seen following the administration of fluoxetine. Whereas the administration of WF-31 alone reduced cerebral metabolism only in the raphe and hippocampus, following antagonism of dopaminergic activity, more widespread decrements in cerebral metabolism, particularly within mesocorticolimbic regions, were evident. These data suggest that the differences between the constellation of alterations in glucose utilization produced by WF-31 and fluoxetine are the result of the additional actions of WF-31 on dopamine systems. The simultaneous actions of WF-31 at both serotonin and dopamine sites observed here are similar to recent findings that the genomic effects of WF-31 in the striatum can be blocked by pretreatment with the dopaminergic antagonist, α-flupenthixol, as well (Daunais et al., 1995, submitted).

Fluoxetine, along with other serotonin selective reuptake inhibitors, is widely accepted as the drug of choice for the treatment of depression and obsessive-compul-

sive disorder. The similarities between the functional effects of fluoxetine and the cocaine analog, WF-31, along with the similarity of their actions in the forced swim test commonly used as a screen for potential antidepressant therapies, suggest that compounds with a cocaine-like structure may be useful in the treatment of psychiatric disorders. Future structure-activity studies are likely to produce compounds with greater selectivity for the serotonin transporter that may make this a fruitful strategy for drug development. Furthermore, the present studies show the usefulness of brain metabolism studies to aid in the assessment of novel pharmacological agents.

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