J. Pharm. Pharmacol. 1996, 48: 68–70
Received February 13, 1995
Accepted August 29, 1995

Serum Corticosterone Increases Reflect Enhanced Uptake Inhibitor-induced Elevation of Extracellular 5-Hydroxytryptamine in Rat Hypothalamus

RAY W. FULLER, KENNETH W. PERRY, SUSAN K. HEMRICK-LUECKE AND ERIC ENGLEMAN

Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Centre, Indianapolis, IN 46285, USA

Abstract

The increase in extracellular 5-hydroxytryptamine (5-HT) in rat hypothalamus following administration of fluoxetine, a 5-HT-uptake inhibitor, was enhanced by the injection of LY206130 (1-[1-H-indol-4-yloxy]-3-[cyclohexylamino]-2-propanol maleate), a $5HT_{1A}$ receptor antagonist, or by L-5-hydroxytryptophan (L-5-HTP), the 5-HT precursor. Elevation of serum corticosterone, measured as a functional output of hypothalamic 5-HT pathways, was greater in rats treated with fluoxetine plus LY206130 or with fluoxetine plus L-5-HTP than in rats treated with the agents alone.

Synergism between effects of fluoxetine and L-5HTP has often been reported, but this is the first report of an increased functional effect when a $5-HT_{1A}$ receptor antagonist is combined with a 5-HT uptake inhibitor to augment the increase in extracellular 5-HT.

The inhibition of 5-hydroxytryptamine (5-HT) uptake by fluoxetine and other selective compounds results in increased concentration of extracellular 5-HT in various brain regions (Perry & Fuller 1993; Fuller 1994), and in increased 5-HT function indicated by behavioural and other effects (Fuller 1992a). Extracellular 5-HT concentration is determined by the rate of neuronal release of 5-HT and the rate of removal of 5-HT from the extracellular space, the removal occurring mainly through uptake by the membrane transporter. When uptake is inhibited and extracellular 5-HT accumulates, the increased activation of autoreceptors on 5-HT neurons diminishes 5-HT release and limits the increase in extracellular 5-HT (Fuller 1994; Hjorth & Auerbach 1994). Extracellular 5-HT is not increased as much by uptake inhibitors as it is by 5-HT-releasing drugs, which release 5-HT independently of nerve firing (Kalen et al 1988; Sabol et al 1992; Fuller 1994).

Recently the antagonism of $5HT_{1A}$ autoreceptors has been shown to enhance the increase in extracellular 5-HT resulting from uptake inhibition (Hjorth 1993). Coadministration of the 5-HT precursor, L-5-hydroxytryptophan (L-5HTP), has also been shown to enhance the increase in extracellular 5-HT resulting from uptake inhibition (Perry & Fuller 1993). In the latter case, the larger increase in extracellular 5-HT by the combination of L-5HTP plus fluoxetine is associated with many functional effects that are larger after the drug combination than after the uptake inhibitor alone (Fuller et al 1975b, 1976; Perry & Fuller 1993). To date, there have been no reports that 5-HT function is increased more by the combination of an uptake inhibitor with a $5HT_{1A}$ receptor antagonist than by an uptake inhibitor alone. To study that possibility, we measured one functional effect of 5-HT neurons, their stimulatory input to the pituitary-adrenocortical axis (Fuller 1992b).

5-HT nerve terminals in the hypothalamus make synaptic contact with corticotropin releasing factor (CRF)-containing neurons (Liposits et al 1987). Direct- and indirect-acting 5-HT receptor agonists increase CRF release into the portal circulation to the anterior pituitary gland (Gibbs & Vale 1983) and increase ACTH and corticosterone in the systemic circulation in rats (Fuller 1992b). Previously we had shown that fluoxetine and L-5HTP acted synergistically to increase serum corticosterone concentration in rats (Fuller et al 1975b; 1976), just as they acted synergistically to increase extracellular 5-HT concentration in rat hypothalamus (Perry & Fuller 1993). Here we describe studies of a $5HT_{1A}$ receptor antagonist combined with fluoxetine, which resulted in a similar synergistic effect on serum corticosterone concentration and on extracellular 5-HT concentration in hypothalamus.

In a published study of $5HT_{1A}$ receptor antagonism combined with uptake inhibition, Hjorth (1993) used the β-adrenergic-receptor-blocking drug penbutolol which also antagonizes $5HT_{1A}$ receptors. In the current study, we used an analogue of pindolol, another β-blocker which is also a $5HT_{1A}$ receptor antagonist. This pindolol analogue, LY206130 (Wong et al 1994), has preferential affinity for $5HT_{1A}$vs β-adrenergic receptors.

Methods

Male Sprague-Dawley rats, 190–300 g, were purchased from Charles River Breeding Laboratories, Portage, MI. Fluoxetine hydrochloride and LY206130 (1-[1-H-indol-4-yloxy]-3-[cyclohexylamino]-2-propanol maleate) were synthesized in the Lilly Research Laboratories. L-5HTP was purchased from Sigma Chemical Company, St Louis, MO. Microdialysis experiments in conscious, freely moving rats and measurement of 5-HT concentrations by liquid chromatography

Correspondence: R. W. Fuller, Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, Indiana 46285, USA.

with electrochemical detection were as described by Perry & Fuller (1992, 1993). The microdialysis probe was implanted stereotaxically in chloral hydrate/pentobarbitone anaesthetized rats. The rats were allowed to recover for two days before use in a microdialysis experiment. Drug doses, vehicles and treatment times were as described below. The microdialysis data were analysed by the nonparametric Wilcoxon rank sum test (Bolton 1984).

For corticosterone measurement, separate groups of rats without microdialysis probes implanted were housed in groups of 5 in a 22°C room with lights on from 0700 to 1900 for 1 week to acclimatize to the environment. Food and water were freely available. Rats were treated and were decapitated between 0900 and 1100 h. Fluoxetine hydrochloride at an intraperitoneal dose of 10 mg kg^{-1} was given 2 h before 5-HT or corticosterone measurements; the injection vehicle was distilled water. L-5HTP and LY206130 were injected subcutaneously at doses of 20 and 5 mg kg^{-1} respectively, 1 h before 5-HT or corticosterone measurements; injection vehicle was 0·01 M HCl, and injection volume was 1 mL kg^{-1} in all cases, and all rats received vehicle or drug at each treatment time. The order of treatments was randomized. Trunk blood was collected and allowed to clot, then serum obtained by centrifugation was stored frozen before analysis. The serum corticosterone concentration was measured by radioimmunoassay (Corticosterone ^{3}H kit, ICN Biomedicals, Inc., Costa Mesa, CA). Samples were diluted according to kit instructions and analysed in duplicate. Correlation coefficient for the standard curve was 0·99. The corticosterone antiserum cross-reacted 6% with desoxycorticosterone and less with other steroids tested. Statistical analyses of corticosterone data were by analysis of variance and group comparison by Scheffe's method.

Results

Table 1 shows extracellular concentrations of 5-HT in the hypothalamus, expressed as percentage of the baseline value. A significant increase in extracellular 5-HT was found after the injection of fluoxetine but not after the injection of LY206130 or the low dose of L-5HTP. Larger increases in extracellular 5-HT were found when fluoxetine and LY206130 or L-5HTP were injected in combination.

Table 1. Effect of LY206130 (5 mg kg^{-1}, i.p.) or L-5HTP (20 mg kg^{-1}, i.p.) given 1 h before measurements were made and 1 h after pretreatment with vehicle or fluoxetine (10 mg kg^{-1}) on 5-HT concentration in microdialysis fluid from hypothalamus and serum corticosterone concentration in rats.

Experiment	n	5-HT (% baseline)[a]	n	Corticosterone (ng mL^{-1})
Vehicle control	18	100 ± 11	5	14 ± 1
+ fluoxetine	11	367 ± 32***	5	91 ± 34
LY206130 + vehicle	3	91 ± 17	5	79 ± 18
+ fluoxetine	5	581 ± 62**†	5	254 ± 41*†
L-5HTP + vehicle	4	151 ± 18	5	52 ± 28
+ fluoxetine	6	705 ± 124***†	5	374 ± 78**††

[a]Basal level of 0·334 pmol mL^{-1} (0·100 – 0·705 pmol mL^{-1}).
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with control; †$P < 0.05$, ††$P < 0.01$ compared with fluoxetine-treated animals.

Table 1 shows the concentration of corticosterone in rat serum. Significant increases in serum corticosterone were found in those groups pretreated with fluoxetine and then given either LY206130 or L-5HTP. Nonsignificant effects were produced by fluoxetine, LY206130 and L-5HTP injected alone. The increase in serum corticosterone was 18-fold in rats receiving fluoxetine + LY206130 and 26-fold in rats receiving fluoxetine + L-5HTP. The magnitude of the increases in extracellular 5-HT paralleled the magnitude of the increases in serum corticosterone.

Discussion

Our results with LY206130 combined with fluoxetine are similar to those reported by Hjorth (1993) from studies of penbutolol combined with citalopram with regard to extracellular 5-HT concentrations. Hjorth (1993) measured extracellular 5-HT in hippocampus, whereas we measured it in hypothalamus. Fluoxetine and citalopram, by inhibiting 5-HT uptake, caused increases in extracellular 5-HT. Those increases were exaggerated when 5-HT$_{1A}$ autoreceptors were blocked by LY206130 or by penbutolol, consistent with evidence that 5-HT$_{1A}$ autoreceptors are activated when extracellular 5-HT is increased, leading to a reduction in the firing of 5-HT neurons and limiting the increase in extracellular 5-HT (Clemens et al 1977; Hjorth & Auerbach 1994). The dose of LY206130 that we used is 10 times the ED50 dose of LY206130 (0·5 mg kg^{-1}, s.c.) for blocking the elevation of serum corticosterone induced by 8-hydroxy-2-(di-*n*-propylamino)tetralin (unpublished data). The dose of fluoxetine was chosen to give complete inhibition of 5-HT uptake based on lowering of brain concentrations of 5-hydroxyindoleacetic acid (Fuller et al 1974) and blockade of brain 5-HT depletion by *p*-chloroamphetamine (Fuller et al 1975a). The dose of L-5HTP was based on a previous publication showing significant enhancement of extracellular 5-HT in fluoxetine-treated rats (Perry & Fuller 1993).

5-HT-uptake inhibitors increase extracellular 5-HT (Fuller 1994) and cause functional effects resulting from increased 5-HT transmission (Fuller 1992a). Those functional effects include decreased muricidal aggression (Molina et al 1987), decreased food intake and altered food preference (Goudie et al 1976; Kim & Wurtman 1988), decreased rapid-eye-movement sleep (Slater et al 1978), anticonvulsant effects in epilepsy models in rats (Dailey et al 1992) and activation of the pituitary-adrenocortical axis (Fuller et al 1976). The increase in extracellular 5-HT after uptake inhibition is limited by reducing firing and release of 5-HT resulting from activation of somatic and terminal autoreceptors on 5-HT neurons, but nonetheless is sustained for the duration of uptake inhibition (Rutter & Auerbach 1993). Uptake inhibition alone does not cause some effects produced by direct-acting 5-HT agonists, by 5-HT-releasing drugs, or by high doses of L-5HTP, presumably because those effects require higher degrees of activation of 5-HT receptors; an example of such an effect is elevation of serum prolactin in rats (Krulich 1975; Clemens et al 1977).

Previous studies have shown that pretreatment with fluoxetine to inhibit 5-HT uptake potentiates many effects of L-5HTP including activation of pituitary-adrenocortical function (Fuller et al 1975b, 1976), reduction of food intake

(Goudie et al 1976), lowering of blood pressure in hypertensive rats (Fuller et al 1979) and anticonvulsant effects in genetically epilepsy-prone rats (Yan et al 1994). Such potentiation agrees well with the exaggerated increase in extracellular 5-HT measured recently by microdialysis in rats treated with fluoxetine plus L-5HTP (Perry & Fuller 1993). Although it has been reported that 5-HT_{1A}-receptor antagonists enhance the increase in extracellular 5-HT caused by uptake inhibition (Hjorth 1993), there has been no functional evidence that 5-HT neurotransmission is increased to a larger extent. The present findings show that LY206130 exaggerates the fluoxetine-induced increase in extracellular 5-HT and that the increased amounts of extracellular 5-HT in rats treated with that combination are associated with increased activation of the pituitary-adrenocortical axis. The increases in extracellular 5-HT and in serum corticosterone concentration were slightly less in the group of rats treated with fluoxetine plus LY206130 than in the group of rats treated with fluoxetine plus L-5HTP.

Acknowledgments

We are grateful to Edward E. Beedle, Joseph H. Krushinski and Dr David W. Robertson for the synthesis and supply of LY206130.

References

Bolton, S. (1984) Pharmaceutical Statistics. Practical and Clinical Applications. Marcel Dekker, New York

Clemens, J. A., Sawyer, B. D., Cerimele, B. (1977) Further evidence that serotonin is a neurotransmitter involved in the control of prolactin secretion. Endocrinology 100: 692–698

Dailey, J. W., Yan, Q. S., Mishra, P. K., Burger, R. L., Jobe, P. C. (1992) Effects of fluoxetine on convulsions and on brain serotonin as detected by microdialysis in genetically epilepsy-prone rats. J. Pharmacol. Exp. Ther. 260: 533–540

Fuller, R. W. (1992a) Basic advances in serotonin pharmacology. J. Clin. Psychiatr. 53 (suppl.) 10: 36–45

Fuller, R. W. (1992b) The involvement of serotonin in regulation of pituitary-adrenocortical function. Front. Neuroendocrinol. 13: 250–270

Fuller, R. W. (1994) Uptake inhibitors increase extracellular serotonin concentration measured by brain microdialysis. Life Sci. 55: 163–167

Fuller, R. W., Perry, K. W., Molloy, B. B. (1974) Effect of an uptake inhibitor on serotonin metabolism in rat brain: studies with 3-(*p*-trifluoromethylphenoxy)-*N*-methyl-3-phenylpropylamine (Lilly 110140). Life Sci. 15: 1161–1171

Fuller, R. W., Perry, K. W., Molloy, B. B. (1975a) Effect of 3-(*p*-trifluoromethylphenoxy)-*N*-methyl-3-phenylpropylamine on the depletion of brain serotonin by 4-chloroamphetamine. J. Pharmacol. Exp. Ther. 193: 796–803

Fuller, R. W., Snoddy, H. D., Molloy, B. B. (1975b) Potentiation of the L-5-hydroxytryptophan-induced elevation of plasma corticosterone levels in rats by a specific inhibitor of serotonin uptake. Res. Commun. Chem. Pathol. Pharmacol. 10: 193–196

Fuller, R. W., Snoddy, H. D., Molloy, B. B. (1976) Pharmacologic evidence for a serotonin neural pathway involved in hypothalamus-pituitary-adrenal function in rats. Life Sci. 19: 337–346

Fuller, R. W., Holland, D. R., Yen, T. T., Bemis, K. G., Stamm, N. B. (1979) Antihypertensive effects of fluoxetine and L-5-hydroxytryptophan in rats. Life Sci. 25: 1237–1242

Gibbs, D. M., Vale, W. (1983) Effect of the serotonin reuptake inhibitor fluoxetine on corticotropin-releasing factor and vasopressin secretion into hypophyseal portal blood. Brain Res. 280: 176–179

Goudie, A. J., Thornton, E. W., Wheeler, T. J. (1976) Effects of Lilly 110140, a specific inhibitor of 5-hydroxytryptamine uptake, on food intake and on 5-hydroxytryptophan-induced anorexia. Evidence for serotoninergic inhibition of feeding. J. Pharm. Pharmacol. 28: 318–320

Hjorth, S. (1993) Serotonin 5-HT_{1A} autoreceptor blockade potentiates the ability of the 5-HT reuptake inhibitor citalopram to increase nerve terminal output of 5-HT in vivo: a microdialysis study. J. Neurochem. 60: 776–779

Hjorth, S., Auerbach, S. B. (1994) Further evidence for the importance of 5-HT_{1A} autoreceptors in the action of selective serotonin reuptake inhibitors. Eur. J. Pharmacol. 260: 251–255

Kalen, P., Strecker, R. E., Rosengren, E., Bjorklund, A. (1988) Endogenous release of neuronal serotonin and 5-hydroxyindoleacetic acid in the caudate-putamen of the rat as revealed by intracerebral dialysis coupled to high-performance liquid chromatography with fluorimetric detection. J. Neurochem. 51: 1422–1435

Kim, S. H., Wurtman, R. J. (1988) Selective effects of CGS 10686B, dl-fenfluramine or fluoxetine on nutrient selection. Physiol. Behav. 42: 319–322

Krulich, L. (1975) The effect of a serotonin uptake inhibitor (Lilly 110140) on the secretion of prolactin in the rat. Life Sci. 17: 1141–1144

Liposits, Z., Phelix, C., Paull, W. K. (1987) Synaptic interaction of serotonergic axons and corticotropin releasing factor (CRF) synthesizing neurons in the hypothalamic paraventricular nucleus of the rat. A light and electron microscopic immunocytochemical study. Histochemistry 86: 541–549

Molina, V., Ciesielski, L., Gobaille, S., Isel, F., Mandel, P. (1987) Inhibition of mouse killing behavior by serotonin-mimetic drugs: effects of partial alterations of serotonin neurotransmission. Pharmacol. Biochem. Behav. 27: 123–131

Perry, K. W., Fuller, R. W. (1992) Effect of fluoxetine on serotonin and dopamine concentration in microdialysis fluid from rat striatum. Life Sci. 50: 1683–1690

Perry, K. W., Fuller, R. W. (1993) Extracellular 5-hydroxytryptamine concentration in rat hypothalamus after administration of fluoxetine plus L-5-hydroxytryptophan. J. Pharm. Pharmacol. 45: 759–761

Rutter, J. R., Auerbach, S. B. (1993) Acute uptake inhibition increases extracellular serotonin in the rat forebrain. J. Pharmacol. Exp. Ther. 265: 1319–1324

Sabol, K. E., Richards, J. B., Seiden, L. S. (1992) Fluoxetine attenuates the DL-fenfluramine-induced increase in extracellular serotonin as measured by in vivo dialysis. Brain Res. 585: 421–424

Slater, I. H., Jones, G. T., Moore, R. A. (1978) Inhibition of REM sleep by fluoxetine, a specific inhibitor of serotonin uptake. Neuropharmacology 17: 383–389

Wong, D. T., Mayle, D. N., DeLapp, N. W., Calligaro, D. O., Robertson, D. W. (1994) LY206130, a cyclohexyl analog of pindolol, an antagonist of 5-HT_{1A} receptor. Soc. Neurosci. Abstr. 20: 1542

Yan, Q.-S., Jobe, P.C., Dailey, J. W. (1994) Evidence that a serotonergic mechanism is involved in the anticonvulsant effect of fluoxetine in genetically epilepsy-prone rats. Eur. J. Pharmacol. 252: 105–112