ELSEVIER

European Journal of Pharmacology 312 (1996) R1–R2

ejp

Rapid communication

Prolactin response to fenfluramine is independent of serotonin release

Una D. McCann [a], George Hatzidimitriou [b], George A. Ricaurte [b,*]

[a] *Unit on Anxiety Disorders, Biological Psychiatry Branch, National Institute on Mental Health, NIH, Building 10 / 3N212; MSC 1272, 10 Center Drive, Bethesda, MD 20892-1272, USA*

[b] *Department of Neurology, 5501 Bayview Boulevard, Johns Hopkins Medical Institutions, Baltimore, MD 21224, USA*

Received 18 July 1996; accepted 30 July 1996

Abstract

To assess the role of serotonin release in the prolactin response to fenfluramine, rats were treated with fenfluramine alone or in combination with a dose of fluoxetine known to block fenfluramine-induced serotonin release. Fluoxetine pretreatment did not prevent fenfluramine-induced increases in prolactin. These findings indicate that fenfluramine-induced increases in prolactin are independent of serotonin release, and possibly involve direct post-synaptic actions of fenfluramine or one of its metabolites (norfenfluramine).

Keywords: 5-HT (5-hydroxytryptamine, serotonin); Fluoxetine; Neuroendocrine challenge

Pharmacological challenge with D,L- or D-fenfluramine has been widely used in neuropsychiatric research as an indirect and relatively non-invasive method for evaluating the dynamic functioning of central serotonin neurons (see Muldoon et al., 1996, for review). Fenfluramine administration has been demonstrated to induce increases in serum prolactin, an effect that has been attributed to indirect serotonergic agonist activity following fenfluramine-induced serotonin release. Recent studies of fenfluramine's behavioral effects indicate that serotonin release is not necessary for fenfluramine-induced anorexia (Raiteri et al., 1995; McCann et al., 1995), and lend support to the proposal that fenfluramine (or a metabolite of fenfluramine) possesses functionally significant direct post-synaptic receptor activity (Gibson et al., 1993). Taken together, these findings raise the possibility that fenfluramine-induced prolactin release might also be mediated via direct post-synaptic receptor action, rather than via serotonin release. Since a clear understanding of the mechanism by which fenfluramine increases serum prolactin is essential for the proper interpretation of fenfluramine challenges in neuropsychiatric research, the present study was performed to assess the role of serotonin release in the prolactin response to fenfluramine.

Male Sprague-Dawley rats (Harlan, Madison, WI, USA; $n = 32$) weighing 200–225 g were housed individually in a temperature-controlled room (22 ± 1°C) on a 12:12 h light/dark cycle (light from 6 a.m. to 6 p.m.), with free access to food (Purina rodent chow) and water. There were four treatment groups: (1) saline plus fenfluramine, 5 mg/kg ($n = 8$); (2) fluoxetine 5 mg/kg plus saline ($n = 8$); (3) fluoxetine 5 mg/kg plus fenfluramine 5 mg/kg ($n = 8$); and (4) saline plus saline ($n = 8$). Both fenfluramine and fluoxetine were given i.p.; fluoxetine was given 30 min prior to fenfluramine. 30 min after fenfluramine, animals were decapitated and trunk blood was collected. Serum prolactin and corticosterone levels were determined using a standard RIA protocol, as previously described (McCann et al., 1994). Neuroendocrine measures (prolactin and corticosterone) were analyzed by one-way ANOVA, with post-hoc Bonferroni tests.

Animals treated with fluoxetine plus saline were found to have serum prolactin levels similar to those treated with saline alone (Fig. 1). As expected, animals treated with fenfluramine plus saline had significant elevations of prolactin. Prolactin levels in animals treated with fluoxetine at doses known to prevent fenfluramine-induced serotonin release (Raiteri et al., 1995) were no different than those in animals treated with fenfluramine alone. Fluoxetine pretreatment also failed to block fenfluramine-induced increases in serum corticosterone (not shown).

The present results strongly suggest that fenfluramine-induced increases in serum prolactin are independent of serotonin release. Recent microdialysis studies clearly establish that pretreatment with fluoxetine (2.5 mg/kg i.p.) completely inhibits fenfluramine-induced serotonin release

* Corresponding author. Tel.: +1 410 550 0993; fax: +1 410 550 2005.

PII S0014-2999(96)00596-1

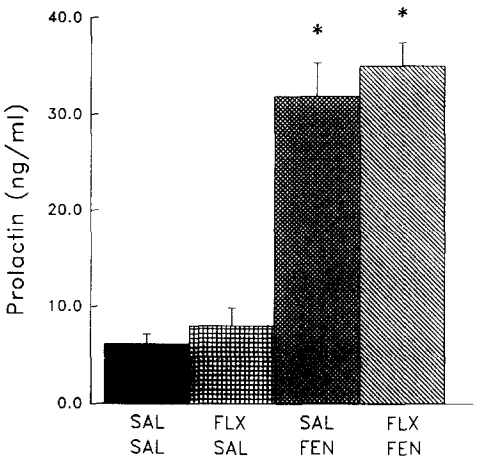

Fig. 1. Plasma prolactin responses to vehicle (SAL) plus fenfluramine (FEN), fluoxetine (FLX) plus fenfluramine, fluoxetine plus vehicle, and vehicle plus vehicle. Drugs were administered i.p. 30 min apart at a dose of 5 mg/kg. Fluoxetine was given first, since under these conditions it is known to prevent fenfluramine-induced serotonin release (Raiteri et al., 1995). 30 min after the second drug treatment (FEN), rats were decapitated and trunk blood was collected for measurements of plasma prolactin using a standard RIA procedure. Data represent mean prolactin concentrations in ng/ml ± S.E.M. ($n = 8$ per group). Results from ANOVA indicate that treatment with vehicle plus fenfluramine or fenfluramine plus fluoxetine lead to similar significant increases in plasma prolactin concentrations. Prolactin concentrations in animals treated with fluoxetine plus vehicle were no different than those in vehicle plus vehicle-treated controls. * $P < 0.05$ compared to control.

(Raiteri et al., 1995). Yet, in the present study, pretreatment with even a higher dose of fluoxetine (5 mg/kg) failed to block fenfluramine-induced increases in serum prolactin (Fig. 1). When considered with other reports that 5-HT_{2C} receptor antagonists totally abolish fenfluramine-induced increases in prolactin (Goodall et al., 1993), as well as data indicating that norfenfluramine (fenfluramine's major metabolite) has activity at 5-HT_{2C} receptors (Gibson et al., 1993), the present findings suggest that fenfluramine-induced increases in prolactin are not mediated by serotonin release but by direct activity at 5-HT_{2C} receptors. This conclusion is also supported by observations from clinical studies that fluoxetine does not block the prolactin response to fenfluramine (Sommers et al., 1994), and that 5-HT_{1A} receptor antagonists do not enhance fenfluramine-induced prolactin release (Park and Cowen, 1995).

Taken together, the available preclinical and clinical data suggest that neuroendocrine challenge tests with D,L- or D-fenfluramine provide a more limited measure of central serotonin neuronal function than heretofore suspected. In particular, it would appear that rather than providing a measure of 5-HT release, the prolactin response to fenfluramine provides a measure of drug-induced 5-HT_{2C} receptor activation (Gibson et al., 1993). The implications of these findings to the interpretation of neuroendocrine challenge tests with fenfluramine in neuropsychiatric patient populations remain to be determined. Finally, the present results add to a growing body of evidence that fenfluramine (via its major metabolite, norfenfluramine), in addition to indirect actions mediated by serotonin release, may produce direct post-synaptic effects.

Acknowledgements

This work was supported by National Institutes of Health Grants PHS R01 DA06275 and K02 DA00206 to G.A.R., and by IRP support to U.D.M.

References

Gibson, E.L., A.J. Kennedy and G. Curzon, 1993, *d*-Fenfluramine and *d*-norfenfluramine-induced hypophagia: differential mechanisms and involvement of post-synaptic 5-HT receptors, Eur J. Pharmacol. 242, 83.

Goodall, E.M., P.J. Cowen, M. Franklin and T. Silverstone, 1993, Ritanserin attenuates anorectic, endocrine and thermic responses to *d*-fenfluramine in human volunteers, Psychopharmacology 112, 461.

McCann, U.D., A. Ridenour, Y. Shaham and G.A. Ricaurte, 1994, Serotonin neurotoxicity after (±)3,4-methylenedioxymethamphetamine (MDMA; 'ecstasy'): a controlled study in humans, Neuropsychopharmacology 10(2), 129.

McCann, U.D., J. Yuan and G.A. Ricaurte, 1995, Fenfluramine's appetite suppression and serotonin neurotoxicity are separable, Eur. J. Pharmacol. 283, R5.

Muldoon, M.F., S.B. Manuck, C.L. Jansma, A.L. Moore, J. Perel and J.J. Mann, 1996, D,L-Fenfluramine challenge test: experience in nonpatient sample, Biol. Psychiatry 39, 761.

Park, S.B.G. and P.J. Cowen, 1995, Effect of pindolol on the prolactin response to *d*-fenfluramine, Psychopharmacology 118, 471.

Raiteri, M., G. Bonanno and F. Vallebuona, 1995, In vitro and in vivo effects of *d*-fenfluramine: no apparent relation between 5-hydroxytryptamine release and hypophagia, J. Pharmacol. Exp. Ther. 273, 643.

Sommers, de K., M. van Wyck and J.R. Snyman, 1994, Dexfenfluramine-induced prolactin release as an index of central synaptosomal 5-hydroxytrypamine during treatment with fluoxetine, Eur. J. Clin. Pharmacol. 46, 441.