Extracellular 5-hydroxytryptamine concentration in rat hypothalamus after administration of fluoxetine plus L-5-hydroxytryptophan

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Abstract—Fluoxetine (10 mg kg^{-1}, i.p.) caused a three- to fourfold increase in extracellular 5-hydroxytryptamine (5-HT) concentration measured by microdialysis in hypothalamus of freely moving rats. The addition of L-5-hydroxytryptophan at 20 or 40 mg kg^{-1}, i.p. doses, magnified the increase in extracellular 5-HT to as much as 16 times basal levels, although these doses of L-5-hydroxytryptophan alone had only small effects on extracellular 5-HT. The increased formation of 5-HT following L-5-hydroxytryptophan administration appears to overcome homeostatic mechanisms that limit the increases in extracellular 5-HT caused by uptake inhibition.

Fluoxetine is a selective inhibitor of the 5-hydroxytryptamine (5-HT) uptake carrier which has been shown to increase extracellular concentrations of 5-HT measured by microdialysis in regions of rat brain (Sabol et al 1991; Perry & Fuller 1992; Dailey et al 1992). Fluoxetine and other selective inhibitors of 5-HT uptake produce several functional effects indicative of increased 5-HT-ergic neurotransmission, e.g. increased concentrations of corticosterone and adrenocorticotrophin in the systemic circulation and of corticotrophin-releasing hormone in hypophyseal portal blood of rats (Fuller et al 1975; Gibbs & Vale 1983), decreased REM sleep in rats and cats (Slater et al 1978) and suppression of muricidal aggression in rats (Kostowski et al 1984; Molina et al 1986). The combination of fluoxetine plus the 5-HT precursor, L-5-hydroxytryptophan, produces exaggerated 5-HT-ergic responses or responses not caused by the uptake inhibitor alone, e.g. greater suppression of food intake (Goudie et al 1976; Fuller & Owen 1981), elevation of serum prolactin (Krulich 1975; Clemens et al 1977) and reduced blood pressure in hypertensive rats (Fuller et al 1979). The present experiments were performed to compare extracellular 5-HT concentrations in rat brain after treatment with fluoxetine and L-5-hydroxytryptophan alone or in combination.

The hypothalamus was chosen as the site of measurement because 5-HT synapses in hypothalamus probably mediate some of the synergistic actions of fluoxetine and 5-hydroxytryptophan that have been demonstrated, such as decreased food intake in meal-fed rats (Goudie et al 1976), decreased milk drinking in nonfasted rats (Fuller & Owen 1981), and activation of the pituitary-adrenocortical axis (Fuller et al 1975). The hypothalamus is an important brain region involved in the regulation of food intake (Leibowitz et al 1988) and in the control of pituitary secretion (Ganong et al 1987) and is a projection area for 5-HT neurons (Liposits et al 1987; Jacobs & Azmitia 1992).

Materials and methods

Fluoxetine hydrochloride was synthesized in the Lilly Research Laboratories and used in the racemic form. L-5-Hydroxytryptophan was purchased from Sigma Chemical Company, St Louis, MO, USA. Male Sprague-Dawley rats, 260-300 g, were purchased from Harlan-Sprague-Dawley, Cumberland, IN, USA. Microdialysis experiments in freely moving rats and measurement of 5-HT concentrations by liquid chromatography with

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electrochemical detection were as described by Perry & Fuller (1992), with the exception that the dialysis loop-type probe was stereotaxically implanted in the right lateral hypothalamus at co-ordinates from the atlas of Paxinos & Watson (1986): rostral—1·5 mm, lateral—1·3 mm, and ventral—9 mm. Correct probe placement was verified at the end of some initial experiments by perfusing a dye (2, 3, 5-triphenyl-2H-tetrazolium chloride, 5 mg mL^{-1}) through the dialysis loop for 10 min and then, after removal, the frozen brain was sectioned with a microtome. In subsequent experiments the brain was dissected and the probe track was observed visually.

Results

Fig. 1 shows the concentration of extracellular 5-HT measured by microdialysis in rat hypothalamus after the injection of fluoxetine followed by two different doses of L-5-hydroxytryptophan. Fluoxetine caused a three- to fourfold increase in extracellular 5-HT concentration which reached a plateau within 2 h. The subsequent injection of L-5-hydroxytryptophan at a 20 mg kg^{-1} intraperitoneal dose caused a further doubling of extracellular 5-HT concentration, and the subsequent injection of L-5-hydroxytryptophan at a 40 mg kg^{-1} intraperitoneal dose increased extracellular 5-HT concentration to 16 times baseline. The lower dose of L-5-hydroxytryptophan alone caused a barely detectable increase in extracellular 5-HT concentration, and the higher dose of L-5-hydroxytryptophan caused a fourfold increase in 5-HT concentration without fluoxetine pretreatment.
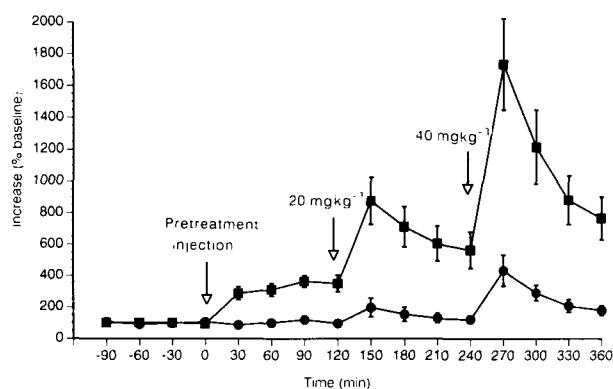

FIG. 1. Increased extracellular concentration of 5-HT measured by microdialysis in hypothalamus of rats given L-5-hydroxytryptophan after pretreatment with fluoxetine (10 mg kg^{-1}, i.p., ■, n=6) or saline (●, n=4). Arrows indicate time of injections. The basal concentrations for the saline and fluoxetine groups were 0·442±0·160 and 0·230±0·046 pmol mL^{-1} dialysate, respectively.

Discussion

The present findings show that the increase in extracellular 5-HT concentration in rat hypothalamus was markedly greater when fluoxetine and L-5-hydroxytryptophan were co-administered

than when either agent was given alone. These findings agree well with functional studies showing that 5-HT-ergic responses produced by fluoxetine alone are exaggerated when L-5-hydroxytryptophan is co-administered, as is the case with an increase in serum corticosterone concentration in rats (Fuller et al 1975, 1976), depression of REM sleep in rats and cats (Slater et al 1978), and suppression of solid food intake in rats (Goudie et al 1976). In addition, there are some effects not produced by fluoxetine alone which are produced by high doses of L-5-hydroxytryptophan or by low doses of L-5-hydroxytryptophan combined with fluoxetine, as is the case with an increase in serum prolactin concentration in rats (Krulich 1975; Clemens et al 1977), head twitch in mice (Ortmann et al 1980), suppression of milk drinking in rats (Fuller & Owen 1981) and lowering of blood pressure in DOCA-salt or spontaneously hypertensive rats (Fuller et al 1979).

Apparently the increase in 5-HT concentration in the synaptic cleft in these latter examples is insufficient after uptake inhibition alone to elicit the response, but the combination of L-5-hydroxytryptophan plus fluoxetine causes larger increases in extracellular 5-HT that do elicit the response. In patients with post-anoxic intention myoclonus, fluoxetine alone had minimum therapeutic effect, but the combination of fluoxetine with a subeffective dose of L-5-hydroxytryptophan was therapeutically effective (Van Woert et al 1983).

L-5-Hydroxytryptophan can be decarboxylated to 5-HT at any site where there is L-aromatic amino acid decarboxylase, an ubiquitous enzyme present not only in 5-HT and catecholamine neurons but in other cells as well. The increased amount of 5-HT in extracellular fluid after L-5-hydroxytryptophan injection does not mean that all of the 5-HT has been released from 5-HT neurons. From a functional point of view, this may not matter, i.e. extracellular 5-HT may affect synaptic receptors whether or not it was released locally into a synaptic gap. However, there has long been histochemical evidence that after low doses in the 20–100 mg kg^{-1} range, 5-hydroxytryptophan is preferentially taken up and decarboxylated within 5-HT neurons, whereas high doses of 5-hydroxytryptophan (500–1000 mg kg^{-1}) are more nonspecific in being converted to 5-HT ubiquitously (see Fuxe et al 1971).

Recently, Gartside et al (1992) have shown that the increase in extracellular 5-HT, as measured by a microdialysis probe in the rat hypothalamus after injection of L-5-hydroxytryptophan, was attenuated by omission of calcium from the perfusion medium, to limit exocytosis. Their finding was interpreted to mean the increased extracellular 5-HT had been released from neurons. Further, the increase in extracellular 5-HT was attenuated by 8-hydroxy-2-(di-n-propylamino) tetralin, a selective agonist at the 5-HT$_{1A}$ subtype of 5-HT receptor. Because the 5-HT$_{1A}$ receptor serves as a somatodendritic autoreceptor on 5-HT neurons and its activation decreases 5-HT neuron firing, the finding suggested that 5-HT neurons, specifically, were the source of the increased extracellular 5-HT following the injection of L-5-hydroxytryptophan. We have found that tetrodotoxin (Perry & Fuller 1992) and 8-hydroxy-2-(di-n-propylamino) tetralin (unpublished data) attenuate the increase in extracellular 5-HT (measured in striatum) elicited by fluoxetine, suggesting 5-HT neuronal release to be the source of the increased extracellular 5-HT produced by this agent as well.

When the uptake carrier on 5-HT neurons is inhibited, the build-up of extracellular 5-HT activates synaptic receptors (pre- or postsynaptically located) to reduce the firing of 5-HT neurons (Clemens et al 1977; Rigdon & Wang 1991) and to suppress 5-HT synthesis and release (Fuller et al 1974; Fuller & Wong 1977). These adaptive mechanisms limit the increase in extracellular 5-HT to approximately three- to fourfold in regions such as hypothalamus (current data), striatum (Perry & Fuller 1992) or

thalamus (Dailey et al 1992) in rats. Thus, homeostatic mechanisms controlling extracellular 5-HT can be overcome by coadministration of L-5-hydroxytryptophan with fluoxetine so that much higher concentrations of extracellular 5-HT are achieved, and functional effects (mentioned above) not produced by uptake inhibition alone may subsequently be seen.

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Platelet activating factor and the responses of rat isolated stomach strip to prostaglandin E_2

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Abstract—The effect of platelet activating factor (PAF) on contrac-
tions evoked by acetylcholine, 5-hydroxytryptamine (5-HT) and
prostaglandin E_2 (PGE_2) was studied in-vitro on rat stomach strip.
Addition of PAF to the organ bath increased PGE- but not 5-HT- or
acetylcholine-evoked responses. The effect of PAF was unaffected by
atropine, methysergide or indomethacin, but prevented by a specific
PAF receptor antagonist BN 52021. The data support a specific
interaction between PAF and PGE_2 on rat stomach strip.

Lyso-PAF and arachidonic acid are precursors of PAF (platelet
activating factor) and prostaglandins, respectively. Both these
precursors are released from membrane-bound phospholipids
by the action of phospholipase A_2. Their common origin could,
in part, explain some biological effects that both prostaglandin
E_2 (PGE_2) and PAF share when released in response to different
stimuli.

However, some of these biological activities, such as local
vasodilatation (Chu et al 1988), hyperalgesia (Dallob et al 1987),
vascular permeability (Oh-ishi et al 1986), pleural exudation
(Martins et al 1989), are modulated by PAF inhibitors and by the
products of the cyclo-oxygenase or lipoxygenase pathways,
indicating an interaction between PAF and arachidonate meta-
bolites. In addition PGE_2 potentiates the inflammatory response
to PAF in animals (Morley et al 1983) and in man (McGivern &
Basran 1984). Furthermore PAF and PGE_2 may regulate the
biosynthesis of one another. We now demonstrate that under
conditions where PAF is itself inactive, it potentiates the effect of
PGE_2, but not of acetylcholine or 5-hydroxytryptamine (5-HT).

Materials and methods

Experiments were carried out in-vitro using stomach strips
(prepared longitudinally) from male Wistar rats, 150-170 g,
killed by cervical dislocation. Strips were mounted in 10 mL
organ baths containing warmed (37°C) and oxygenated (95%
O_2-5% CO_2) Krebs solution of the following composition
(g L^{-1}); NaCl 6·9, KCl 0·35, $MgSO_4 \cdot 7H_2O$ 0·29, KH_2PO_4 0·16,
$CaCl_2$ 0·28, $NaHCO_3$ 2·1, glucose 2·0) and connected to an

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isotonic transducer to record the longitudinal muscle activity.
The resting tension was adjusted to 2 g and maintained
throughout the experiment. The agonists studied were PGE_2
(0·3-2·4 ng mL^{-1}), acetylcholine (5-20 ng mL^{-1}), and 5-HT
(2·5-10 ng mL^{-1}); they remained in contact with the tissue until
the maximal effect occurred (90-120 s) and then washed out.
After at least three control agonist contractions, PAF (12 pg
mL^{-1}) was added to the bath 2 min before the next addition of
agonist. PAF (Sigma, Italy) was dissolved in Tris buffer (pH 7·4)
containing 0·1% bovine serum albumin. In some experiments
the cholinergic antagonist atropine sulphate (0·2 µg mL^{-1}), the
5-HT antagonist methysergide chloride (0·2 µg mL^{-1}), the
prostaglandin synthesis inhibitor indomethacin (2 µg mL^{-1}), or
the PAF antagonist BN 52021 (2 µg mL^{-1}) (Braquet 1985), was
added to the Krebs solution. The results are shown as percent
increase of the respective controls ± s.e. and the raw data were
analysed statistically by Student's *t*-test for paired or unpaired
data (2-tailed tests). The latent period was defined as the time
between the addition of PGE_2 and the start of the contraction.

Results

PGE_2, 0·3-2·4 ng mL^{-1}, caused slow concentration-dependent
contraction of the rat stomach strip with a latent period of 20-
40 s. However, PAF 12 pg mL^{-1}, did not affect the muscle tone
but increased the height of the contraction to PGE_2 (Table 1) and
shortened the latent period to 5-10 s; these effects were totally
removed by washing out the PAF. In contrast, lyso-PAF in
concentrations 12-100 pg mL^{-1} did not affect the response to
PGE_2 (0·3-2·4 ng mL^{-1}) (data not shown). Previous addition of
atropine sulphate (0·2 µg mL^{-1}), methysergide chloride (0·2 µg
mL^{-1}) or indomethacin (2 µg mL^{-1}), alone or in combination, in
concentrations more than sufficient to suppress cholinergic and
5-HT-ergic activities and cyclo-oxygenase activity, did not affect
the potentiating activity of PAF (Table 1). In contrast BN 52021
(2 µg mL^{-1}), a specific PAF receptor antagonist, prevented the
potentiation. The blocking drugs had little or no effect (3-6%
inhibition) on contractions but completely inhibited any sponta-
neous activity that was present. The effect of PAF on agonists
other than PGE_2 was also examined; PAF 12 pg mL^{-1} did not
affect the response to acetylcholine 5-20 ng mL^{-1} or 5-HT 2·5-
10 µg mL^{-1}.