



# Effects of phentermine on striatal dopamine and serotonin release in conscious rats: *In vivo* microdialysis study

A Balcioğlu and RJ Wurtman

Massachusetts Institute of Technology, Department of Brain and Cognitive Sciences, Cambridge MA 02139, USA

**OBJECTIVE:** To measure the effects of phentermine, an appetite suppressant, on the release of brain dopamine (DA) and serotonin (5-HT) into striatal dialysates of freely moving rats.

**DESIGN:** Microdialysis and high performance liquid chromatography.

**SUBJECTS:** Unanesthetized rats.

**MEASUREMENTS:** Samples collected every 20 min were assayed for both neurotransmitters in a single run, using high performance liquid chromatography with electrochemical detection.

**RESULTS:** Baseline levels of DA and 5-HT in dialysates were  $56 \pm 16$  and  $3 \pm 0.6$  fmol/20  $\mu$ l, respectively. Administration of phentermine (2 or 5 mg/kg) increased dialysate DA concentrations to  $147 \pm 17\%$  ( $P < 0.01$ ) and  $320 \pm 89\%$  ( $P < 0.01$ ) of baseline, respectively, without significantly affecting 5-HT concentrations. Pretreatment with tetrodotoxin (TTX, 60 min, 1  $\mu$ M), which abolished the basal release of DA and 5-HT into striatal dialysates, diminished the increase in DA concentrations induced by phentermine, but did not completely block it. Phentermine (2 or 5 mg/kg, i.p.) still stimulated DA release to  $27 \pm 13\%$  and  $85 \pm 15\%$  of baseline, respectively, in the presence of TTX.

**CONCLUSION:** Phentermine increases brain DA but not 5-HT release in freely moving rats, and TTX reduces, but does not fully block this effect. This pattern is similar to that known to be produced by d-amphetamine.

**Keywords:** phentermine; dopamine; serotonin; striatum; microdialysis

## Introduction

The anorectic drug phentermine, introduced into the USA in the 1970's, was thought to act, like its analog d-amphetamine, as a 'sympathomimetic agent with stimulant properties' probably mediated by the catecholamines, i.e. noradrenaline.<sup>1</sup>

That phentermine's action might also involve dopamine (DA) was suggested the finding that its anorectic activity could be blocked by pimozone, a DA receptor antagonist,<sup>2</sup> or by pretreatment with 6-hydroxydopamine, a toxin which damages both noradrenaline and DA brain neurons.<sup>3</sup> However, no direct evidence is available, even now, that phentermine increases intrasynaptic DA levels or enhances DA-mediated neurotransmission. An effect on catecholamine release presumably mediates both the anorectic effect and the numerous side-effects of the structurally-related drug d-amphetamine,<sup>1,4,5</sup> but not of dexfenfluramine, which directly releases serotonin (5-HT) but not DA.

In 1984, Weintraub and associates<sup>6</sup> combined phentermine with another antiobesity drug, fenfluramine, in the hope of reducing side effects observed when each compound was given separately. They found that

the stimulant effects of phentermine and the sedative action of fenfluramine were, in fact, diminished when the drugs were given in combination.<sup>6,7</sup> Fenfluramine is, in fact, two chemicals, i.e. the dextro isomer, which enhances 5-HT-mediated neurotransmission by blocking 5-HT re-uptake<sup>8</sup> and, through its metabolite dexnorfenfluramine, enhancing 5-HT release and activating 5-HT receptors,<sup>9</sup> and l-fenfluramine, which acts like haloperidol, a DA receptor antagonist.<sup>10</sup> Hence combining phentermine with the l-fenfluramine component of fenfluramine might be expected to neutralize the pro- and antidopaminergic activities of the two drugs, if indeed phentermine is a dopaminergic compound.

We now show that phentermine administration to intact, awake rats increases brain DA release. This effect is shared with d-amphetamine, but not with dexfenfluramine.

## Experimental procedures

### Animals

Male Sprague Dawley rats weighing 200–300 g were purchased from Taconic Farms (Germantown, NY); housed two per cage; kept on a 12:12 hr light/dark cycle; and given *ad libitum* access to food and water.

Correspondence: Dr RJ Wurtman, MIT E25-604, Cambridge MA 02139, USA.

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### Drug treatments

Phentermine, dissolved in saline, was administered intraperitoneally. Tetrodotoxin (TTX) was dissolved in artificial cerebrospinal fluid (ACSF). All pharmacological treatments were performed after stabilization of DA and 5-HT levels in perfusate, usually after about 90 min of perfusion. TTX (1  $\mu$ M) was added to perfusates 60 min before animals received phentermine (1, 2 or 5 mg/kg, i.p.). Control animals received only the saline vehicle i.p., or the ACSF perfusate.

### Brain microdialysis

Dialysis probes of a concentric design<sup>11</sup> were constructed from fused silica capillary tubing (Polymicro Technologies, Phoenix, AZ). Tubing of 140  $\mu$ m diameter was inserted within wider silica capillary tubing (300  $\mu$ m) and secured at the inflow site using epoxy resin. A 4 mm length of hollow microdialysis fiber (Spectrum Medical Industries, CA) was sealed at its tip and secured between the end of the wider silica capillary tubing and the inner tubing, using a cyanoacrylate adhesive. As anticipated from the literature and from prior studies in our laboratory, the probes exhibited *in vitro* recoveries of  $11 \pm 2\%$  and  $15 \pm 2\%$  for DA and 5-HT; data were not corrected for probe recovery. Probes were implanted into the striatum (A, +0.7; L, 0.3; V, -6.5; with respect to bregma<sup>12</sup>) of rats that had been anesthetized with ketamine/xylazine (87/13%; 1 ml/kg, i.p.). Animals were used two days after surgery. The probes were perfused at a flow rate of 1.5  $\mu$ l/min, using a CMA microperfusion pump (Carnegie Medicin, Acton, MA), with ACSF (in millimolar): Na<sup>+</sup>, 145; K<sup>+</sup>, 2.7; Mg<sup>++</sup>, 1.0; Ca<sup>++</sup>, 1.2, adjusted to pH  $7.4 \pm 0.2$  with phosphate buffer, 2.0. Samples collected every 20 min were analyzed immediately by high performance liquid chromatography (HPLC)-electrochemical detection. At the end of each experiment, animals were decapitated and probe locations were confirmed by visual examination of probe tracks.

### HPLC with electrochemical detection

Dialysate samples were analyzed by reversed-phase HPLC coupled with electrochemical detection. The mobile phase was composed of 75 mM sodium dihydrogen phosphate (NaH<sub>2</sub>PO<sub>4</sub>), 1 mM sodium dodecyl sulfate (SDS), 0.1 mM ethylenediamine-tetraacetic acid (EDTA), 15% acetonitrile and 20% methanol; this was adjusted to pH 5.7 with sodium hydroxide. This mobile phase was delivered at a flow rate of 1 ml/min (LC-10AD pump, Shimadzu, Columbia, MD) through an HR-80 column (C18, 4.6  $\times$  80 mm, 3  $\mu$ m, ESA, Bedford, MA). DA and 5-HT were detected using a coulometric detector (Coulchem II, ESA) coupled to a dual electrode analytical cell (model 5014). The potential of the first electrode was set at -175 mV, and that of the second at +175 mV. Under these conditions, the sensitivities for DA and 5-HT were 2 fmol/20  $\mu$ l.

### Statistical analysis

The DA and 5-HT contents of each dialysate sample were expressed as a percent of baseline. Data represent means  $\pm$  s.e.m. Effects of the different treatments were analyzed by one way analysis of variance (ANOVA). When significant effects were found, *post hoc* between comparisons were carried out with Dunnett's test.

### Reagents

The reagents used were phentermine and TTX (Sigma Chemical Co. MO, USA); NaH<sub>2</sub>PO<sub>4</sub>, SDS and EDTA (Fluka, Buchs, Switzerland); methanol, acetonitrile (EM Science, Cherry Hill, NJ, USA). Other reagents used were of the highest grade commercially available.

## Results

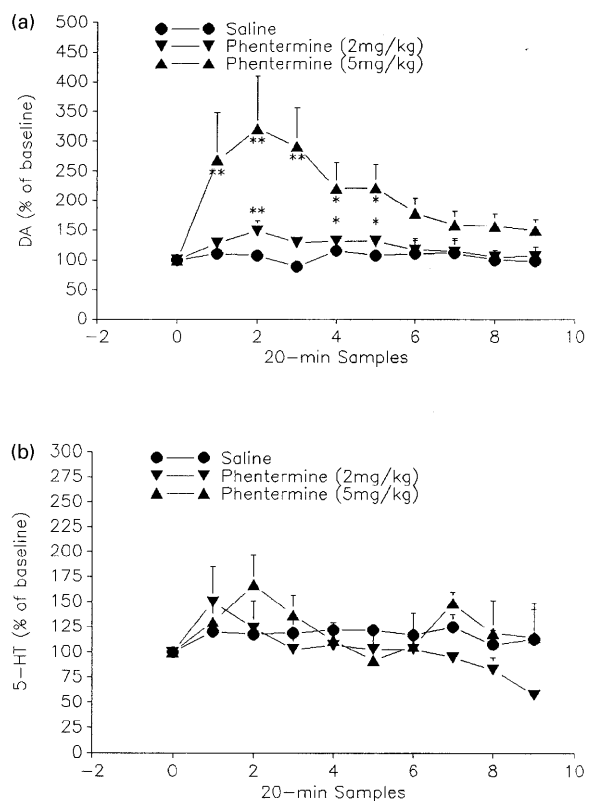
### Effect of a single dose of phentermine on DA and 5-HT levels in dialysates

Baseline DA and 5-HT levels in striatal dialysates collected prior to drug administration were  $56 \pm 16$  and  $3 \pm 0.6$  fmol/20  $\mu$ l ( $n = 25$ ). A one mg/kg phentermine did not affect dialysate DA or 5-HT levels (data not shown). Higher doses (2 or 5 mg/kg) significantly increased DA levels, but not those of 5-HT (Figure 1a and b). The maximum increases were  $148 \pm 17$  ( $P < 0.01$ ) and  $320 \pm 89\%$  of baseline ( $P < 0.01$ ), respectively (Figure 1a). The increase in DA levels reached statistical significance in the 20–40 min sample at 2 mg/kg, and in the 0–20 min sample at 5 mg/kg. DA returned to baseline levels in 2 h.

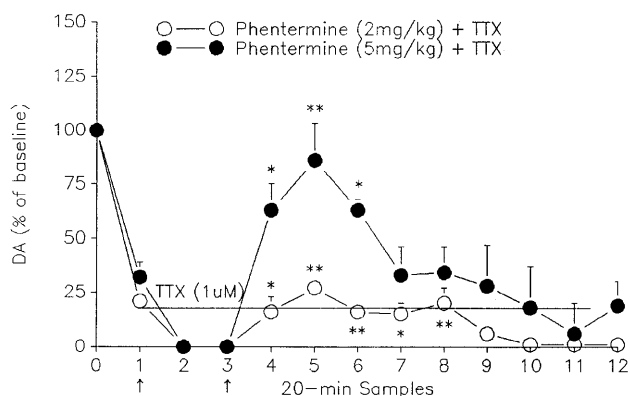
### Effect of TTX on the increases in DA levels induced by phentermine

To determine whether phentermine releases DA by a mechanism similar to that of amphetamine, a related compound, we examined the effect of the sodium channel blocker TTX, administered through the dialysis probe 60 min before phentermine administration and continuously thereafter. We and others had previously shown that tetrodotoxin does not completely block amphetamine-induced DA release,<sup>13,14</sup> implying that at least part of the amphetamine's effect is *via* a carrier-mediated mechanism.

TTX diminished the increase in DA concentrations induced by phentermine, but did not completely block this increase. TTX given alone, reduced baseline DA (Figure 2) and 5-HT (data not shown) release to unmeasurable levels. Administration of phentermine, 60 min after starting local application of TTX, still significantly stimulated DA release into the dialysates (Figure 2). Maximum release in the presence of TTX was to  $27 \pm 13\%$  and  $85 \pm 15\%$  of baseline levels, respectively, at the 2 or 5 mg/kg phentermine doses.



**Figure 1** Effect of phentermine on the release of Dopamine (DA) and serotonin (5-HT) into rat striatal dialysates. After collection of three baseline samples (20 min each), phentermine was administered *i.p.* and sample collection continued for the next 3 h. Each point represents the mean of samples collected during the prior 20 min. Data points were graphed as means  $\pm$  S.E.M. over time.  $n=4-5$ . \* $P < 0.05$ ; \*\* $P < 0.01$ .



**Figure 2** Effect of tetrodotoxin (TTX, a sodium channel blocker, 1  $\mu$ M) on the release of dopamine (DA) induced by phentermine (2 or 5 mg/kg, *i.p.*). TTX, dissolved in the perfusion medium, was applied through the dialysis probe starting 60 min before (first arrow) the administration of phentermine (second arrow), and continuing throughout the experiment. Each point represents the mean of samples collected during the prior 20 min. Data were graphed as means  $\pm$  S.E.M.  $n=4-6$ . \* $P < 0.05$ ; \*\* $P < 0.01$ .

These increases correspond to  $6 \pm 1$  ( $P < 0.01$ ) and  $39 \pm 15$  fmol/20  $\mu$ l ( $P < 0.01$ ), respectively.

## Discussion

These data show that phentermine increases DA release in rat striatum without significantly affecting

that of 5-HT at the doses tested. A related antiobesity drug, amphetamine, has also been shown to increase DA release at relatively lower doses (1 mg/kg, *s.c.*)<sup>15</sup> and to affect 5-HT release at higher doses (2 mg/kg, *i.p.*)<sup>16</sup>. Dexfenfluramine causes 5-HT release at low doses (0.5–1.0 mg/kg, *i.p.*), however at doses above 1.0 mg/kg, this 5-HT secondarily enhances DA release.<sup>17</sup> TTX treatment blocks the release by dexfenfluramine of 5-HT and, thereby, the release of DA, but does not block the direct release of DA by phentermine (Figure 2) or amphetamine.<sup>14</sup> The fact that tetrodotoxin does not completely block DA release induced by amphetamine or phentermine implies that at least part of the effect is via a carrier-mediated mechanism.

Apparently no previous publication has described the effect of phentermine on neurotransmitter release *in vivo*, possibly because phentermine is an 'old' drug and most studies of its mechanism of action were done before *in vivo* microdialysis was introduced.<sup>11</sup> It has been suggested, based on indirect evidence, that phentermine elicits its antiobesity effect *via* catecholaminergic systems, since pimozide, a dopamine receptor blocker, blocked phentermine anorexia in the mouse<sup>2</sup> and 6-hydroxydopamine lesions, which destroy DA and noradrenaline terminals,<sup>18</sup> significantly antagonized the anorectic effect of phentermine.<sup>3</sup> However, no measurements were made of actual DA or noradrenaline levels, and neither pimozide nor 6-hydroxydopamine is entirely specific for DA terminals. Hence this paper presents the first direct evidence of the amphetamine-like properties of phentermine.

Phentermine and fenfluramine have been combined in the hope of reducing the side effects observed (excitation, sedation) when each compound is given separately.<sup>6</sup> Fenfluramine is actually a racemic compound.<sup>19</sup> Analysis of the pharmacological and biochemical effects of its two isomers has shown that dexfenfluramine acts *via* 5-HT mechanisms and suppresses food intake<sup>8</sup> while l-fenfluramine, by blocking DA receptors and diminishing DA-mediated neurotransmission, secondarily increases DA release, much as haloperidol, another dopamine receptor antagonist, does.<sup>10</sup> Since, as we now show, phentermine directly enhances DA release and thus the activation of DA receptors, the phentermine and l-fenfluramine probably do counteract each others' effect on DA transmission. The dopaminergic effect of phentermine might also be expected to produce side-effects similar to those of the prototype dopaminergic anorectic, d-amphetamine.

In earlier studies of brain neurotransmitter release utilizing *in vivo* microdialysis, it was often necessary to measure not the neurotransmitter itself, but its inactive metabolites, because available assays lacked adequate sensitivity. Now that these assays have improved and the neurotransmitters can be measured, it is apparent that treatments can affect neurotransmitter release without necessarily producing parallel

changes in metabolite levels.<sup>20–23</sup> This probably reflects the fact that re-uptake, and not metabolism, is the means whereby such neurotransmitters are inactivated. For this reason, we do not measure levels of DA or 5-HT metabolites in studies on dopaminergic or serotonergic neurotransmission.

In conclusion, phentermine increases striatal DA release in freely moving rats, and TTX reduces, but does not fully block this effect.

### Acknowledgements

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