HMDS; combined therapy with fenclorac and phenobarbital provided protection for both the articular (inflammation) and extra-articular (impairment of HMDS) manifestations of adjuvant disease.

The clinical implications of adjuvant-induced impairment in HMDS and the potential use of combined therapy with an enzyme inducer and nonsteroidal anti-inflammatory agent must be considered in relation to changes in the clinical pharmacological profile of the drug substances. Enzyme induction and drug interaction could significantly alter therapeutic and toxicological activity by changing the rates of metabolism and clearance of the anti-inflammatory drug substance.

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Effects of the *d*- and *l*-isomers of amphetamine on the levels of 3-methoxy-4-hydroxyphenylglycol sulfate in whole rat brain and rat brain regions

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Considerable evidence has accumulated to suggest that amphetamine increases the availability of the catecholamines (norepinephrine and dopamine) at post-synaptic receptors by releasing catecholamines from pre-synaptic nerve terminals or by blocking their reuptake. Aspects of this extensive literature have been reviewed in several recent volumes [1–3]. However, studies of the effects of acute administration of amphetamine or methamphetamine on the turnover of norepinephrine have yielded variable results. In some studies, norepinephrine turnover in whole brain or brain regions has been found to be increased by amphetamine, as determined by the rate of disappearance of intracerebrally injected [^{3}H]norepinephrine [4–7], while in others norepinephrine turnover in brain appeared to be decreased or unchanged as determined by the accumulation of norepinephrine synthesized *in vivo* from labeled precursors [8–10] or by the rate of depletion of endogenous norepinephrine after synthesis inhibition [11].

The methods used in previous studies to examine the effects of amphetamine on norepinephrine turnover involved various pharmacological or physiological interventions. Therefore, in order to explore this problem further, we have examined the effects of both the *d*- and *l*-isomers of amphetamine on the turnover of norepinephrine in whole rat brain and various regions of rat brain by measuring the endogenous levels of the sulfate conjugate of 3-methoxy-4-hydroxyphenylglycol (MHPG-SO_4), a major metabolite of norepinephrine in rat brain [12, 13]. This

has previously been shown to be a useful technique for estimating norepinephrine turnover [14].

Male Sprague–Dawley rats weighing 200–250 g were used in these experiments. In all experiments, *d*- or *l*-amphetamine sulfate was administered by i.p. injection 2 hr prior to sacrifice. (All doses are expressed as the sulfate salt of these isomers.) Control animals received saline injections. In studies of brain regions, the sectioning and removal of the hypothalamus, corpus striatum, cortex, cerebellum, brainstem (pons-medulla) and "rest of brain" were performed using a modification of the method of Glowinski and Iversen [15]. Brain tissue was weighed, frozen in dry-ice methanol and stored prior to assay at −80°.

MHPG-SO_4 was estimated by the method of Meek and Neff [14] with minor modifications [16]. The tissues from three animals were pooled for assays of each brain region. For technical reasons relating to the assay, only the right cerebral cortex was utilized in these experiments. Student's *t*-test was used to determine the statistical significance of differences between experimental and control values.

In order to determine the relative effects of the *d*- and *l*-isomers of amphetamine on MHPG-SO_4 levels in whole brain, dose–response relationships were examined for both isomers. Data presented in Table 1 indicate that the acute administration of *d*-amphetamine increased whole brain levels of MHPG-SO_4 in a dose-related manner, attaining statistical significance at 2.5 and 5 mg/kg. However, increasing the dose of *d*-amphetamine to 10 mg/kg attenuated the increase in MHPG-SO_4 levels that was observed with the lower doses.

As shown in Table 1, *l*-amphetamine appeared to be less potent than *d*-amphetamine with respect to its effect on MHPG-SO_4 levels in rat brain. While significant differences in MHPG-SO_4 levels were not observed after *l*-amphetamine was administered in doses of 2.5 or 5 mg/kg, a significant increase in MHPG-SO_4 levels was observed after *l*-amphetamine was administered in a dose of 10 mg/kg but not after 20 mg/kg.

Using the respective doses of *d*- and *l*-amphetamine that produced significant increases in MHPG-SO_4 levels in whole brain, we examined the effects of these isomers on MHPG-SO_4 levels in various brain regions, including the cortex, hypothalamus, corpus striatum, cerebellum, brainstem and "rest of brain". The findings of these experiments showed that the effects of the *d*- and *l*-isomers of amphetamine on MHPG-SO_4 levels are not uniform throughout all brain regions.

When the *d*-isomer of amphetamine was administered at a dose of 2.5 mg/kg 1 hr prior to sacrifice, a small increase in levels of MHPG-SO_4, which did not reach statistical significance ($0.10 > P > 0.05$), was observed in the hypothalamus, and no other significant changes in levels of MHPG-SO_4 were observed in the cortex, corpus striatum, cerebellum, brainstem or "rest of brain". When *d*-amphetamine (2.5 mg/kg) was administered 2 hr prior to sacrifice, levels of MHPG-SO_4 were significantly increased in the hypothalamus and "rest of brain" but not in the corpus striatum, cerebellum or brainstem (Table 2). A higher dose of *d*-amphetamine (5 mg/kg) administered 2 hr before sacrifice produced a significant increase in levels of MHPG-SO_4 in the cortex as well as in the hypothalamus and "rest of brain"; however, as shown in Table 2, an increase in levels of MHPG-SO_4 in the cortex was not observed after the lower dose (2.5 mg/kg).

Table 1. Effects of *d*- or *l*-amphetamine on MHPG-SO_4 levels in rat brain—dose response*

Dose (mg/kg)	MHPG-SO_4 (pmoles/g brain) *d*-Amphetamine	*l*-Amphetamine
0	463 ± 14	465 ± 18
1.25	490 ± 19	420 ± 16
2.5	532 ± 12†	491 ± 24
5	552 ± 17†	489 ± 19
10	508 ± 34	528 ± 22‡
20		508 ± 18

* The *d*- and *l*-isomers of amphetamine sulfate were administered i.p. 2 hr prior to sacrifice. Results are expressed as means ± S.E.M. of at least seven determinations.

† $P < 0.005$, when compared to respective control.

‡ $P < 0.05$, when compared to respective control.

Table 2. Effects of *d*-amphetamine (2.5 mg/kg) on MHPG-SO_4 levels in rat brain regions*

	MHPG-SO_4 (pmoles/g brain) Control	*d*-Amphetamine	P
Cortex	403 ± 24	388 ± 13	NS
Hypothalamus	684 ± 58	852 ± 54	< 0.05
Corpus striatum	337 ± 34	369 ± 45	NS
Cerebellum	139 ± 16	172 ± 9	NS
Brainstem	513 ± 38	559 ± 28	NS
"Rest of brain"	591 ± 22	655 ± 21	< 0.05

* The *d*-isomer of amphetamine sulfate (2.5 mg/kg) was administered i.p. 2 hr prior to sacrifice. Results are expressed as means ± S.E.M. of at least eight determinations. NS = not significant.

The *l*-isomer of amphetamine (10 mg/kg), administered 2 hr before sacrifice, significantly increased the levels of MHPG-SO_4 in the hypothalamus and "rest of brain" but not in the cerebellum or brainstem (Table 3). A small but statistically nonsignificant increase in MHPG-SO_4 levels was also observed in cortex after the administration of *l*-amphetamine (10 mg/kg) (Table 3).

In the present study, we examined the effects of both the *d*- and *l*-isomers of amphetamine on MHPG-SO_4 levels in whole rat brain and various regions of rat brain. Acute administration of *d*-amphetamine resulted in a dose-related increase in the levels of MHPG-SO_4 in whole rat brain (up to a dose of 5 mg/kg), whereas the *l*-isomer appeared less potent. Similarly, in a recent study Bareggi *et al.* [17] reported that acute administration of *d*-amphetamine (5 mg/kg) produced a significant increase in MHPG-SO_4 levels in whole rat brain. However, in another study, Calderini *et al.* [18] found increased levels of MHPG-SO_4

Table 3. Effects of *l*-amphetamine (10 mg/kg) on MHPG-SO_4 levels in rat brain regions*

	MHPG-SO_4 (pmoles/g brain) Control	*l*-Amphetamine	P
Cortex	392 ± 20	426 ± 21	NS
Hypothalamus	642 ± 40	889 ± 66	< 0.005
Cerebellum	139 ± 12	140 ± 12	NS
Brainstem	643 ± 45	619 ± 36	NS
"Rest of brain"	627 ± 50	800 ± 26	< 0.005

* The *l*-isomer of amphetamine sulfate (10 mg/kg) was administered i.p. 2 hr prior to sacrifice. The corpus striatum was not assayed in this experiment. Results are expressed as means ± S.E.M. of at least eleven determinations. NS = not significant.

in rat brain only after 15 mg/kg of *d*-amphetamine, a dose higher than that required in our studies or those of Bareggi *et al.* [17]. In another recent study, Peterson and Sparber [19], examining the effects of *d*- and *l*-amphetamine on the release and metabolism of intraventricularly infused [^{3}H]norepinephrine in rat brain, found that *d*-amphetamine (3 mg/kg) produced a slight but statistically nonsignificant increase in levels of tritiated MHPG in perfusates from the lateral ventricles, while *l*-amphetamine (6 mg/kg) produced a statistically significant increase in levels of tritiated MHPG.

Thus, it appears that, when administrated in sufficient doses, both isomers of amphetamine increase the turnover of norepinephrine, as reflected by increased levels of MHPG-SO_4 in whole rat brain. The findings of this study, using levels of MHPG-SO_4 as an index of norpinephrine turnover, are consistent with the results of earlier isotopic studies in which amphetamine was found to increase the turnover of norepinephrine—as determined by measuring the rate of disappearance of intracerebrally administered [^{3}H]norepinephrine from the brain [5–7]—but they do not appear to be consistent with the results of studies in which norepinephrine turnover was determined by measuring the accumulation of norepinephrine synthesized *in vivo* from labeled precursors [8–10]. However, interpretation of the findings of studies measuring the accumulation of norepinephrine synthesized from labeled precursors is complicated by the possibility that amphetamine may preferentially release newly synthesized catecholamines [10].

While conflicting findings have emerged from previous studies comparing the relative potencies of *d*- and *l*-amphetamine on norepinephrine uptake in brain [20–24], the present results indicate that the *d*-isomer of amphetamine is two to four times more potent than the *l*-isomer in increasing the levels of MHPG-SO_4 in whole rat brain. Similarly, several studies have indicated that the *d*-isomer of amphetamine is two to three times more potent than the *l*-isomer in maintaining amphetamine self-administration in rats [25] and five times more potent in dogs [26] or in monkeys [27]. Studies in man indicate that *d*-amphetamine is two to four times more potent than *l*-amphetamine in producing euphoric and antidepressant effects [28]. In clinical studies of a small group of amphetamine users, increased urinary MHPG excretion was observed during self-administration of relatively high doses when patients were clinically hypomanic, whereas a reduction in MHPG excretion was observed after amphetamine withdrawal when patients were depressed [29]. While these studies, considered together, suggest that noradrenergic neuronal systems may play a role in the reinforcing and euphoriant properties of amphetamines [30, 31], other neurotransmitters, including dopamine, may also be involved in the mediation of these effects [1–3, 32].

It is of interest to note that the highest dose of each isomer, 10 mg/kg of *d*-amphetamine and 20 mg/kg of *l*-amphetamine, attenuated the increases in MHPG-SO_4 levels which were observed with lower doses. This could conceivably be due to an amphetamine-induced inhibition of monamine oxidase, since this enzyme is required for the formation of MHPG [13], although other possibilities cannot be excluded.

The doses of the amphetamine isomers that were employed in the regional studies were those that produced significant changes in the levels of MHPG-SO_4 in whole brain. In these studies, both doses of *d*-amphetamine (2.5 or 5 mg/kg) significantly increased the levels of MHPG-SO_4 in the hypothalamus and "rest of brain" but not in the cerebellum or the brainstem. The magnitude of increase in MHPG-SO_4 in the hypothalamus (i.e. approximately 25 per cent) after *d*-amphetamine (2.5 mg/kg) suggests that this effect may occur at even lower doses of the drug. In the cortex, an increase in MHPG-SO_4 was observed only after a larger dose of *d*-amphetamine (5 mg/kg). Similarly, *l*-amphetamine (10 mg/kg) significantly increased norepinephrine turnover in the hypothalamus and "rest of brain" but not in the cerebellum or brainstem. In the cortex, *l*-amphetamine (10 mg/kg) produced a small, statistically nonsignificant increase in MHPG-SO_4 levels.

Thus, the two amphetamine isomers, although differing in potency, tended to produce qualitatively similar effects on norepinephrine turnover in the various regions of the rat brain that we examined. Among other possibilities, it is conceivable that the regional differences in the effects of *d*- and *l*-amphetamine on the levels of MHPG-SO_4 could have resulted from differences in levels of amphetamine in various brain regions. Further studies will be required to explore this problem.

In comparison to the effects of amphetamine observed in this study, morphine (25 mg/kg) has been found to increase the levels of MHPG-SO_4 in the cerebellum and brainstem as well as in the hypothalamus and "rest of brain" but not in the cortex or corpus striatum [33, 34]. These findings on the effects of morphine and amphetamine on norepinephrine turnover in various brain regions may help to account for some of the similarities and differences in the effects of these drugs on aspects of behavior, affect and mental state.

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