BEHAVIOURAL PHARMACOLOGY OF D-AMPHETAMINE: SOME METABOLIC AND PHARMACOLOGICAL CONSIDERATIONS

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FOOD deprivation and amphetamine are both known to increase behavioural arousal in the rat. Amphetamine is generally believed to induce psychomotor excitation by increasing the synaptic release of catecholamines (CA) and by blocking their reuptake from the synapse.[7,13,30] Food deprivation increases electrophysiological measures of arousal in the mesencephalic reticular formation and other brain regions[26] but the neurochemical substrates of this response are presently not known. DELL[10] postulated that the behavioural arousal induced by starvation resulted from the release of adrenal catecholamines and their subsequent action on excitatory mechanisms in the brain stem. This hypothesis has not been supported, however, by the finding that rats continue to show starvation-induced arousal after adrenal demedullation.[14] Recently, the question of how starvation-induced and amphetamine-induced increases in behavioural arousal might interact was investigated.[6] The stimulant effects of amphetamine were found to interact synergistically with starvation-induced arousal. Thus, food-deprived rats were significantly more responsive to the stimulant effects of amphetamine than were controls. In addition, the response to a given dose of amphetamine (1 mg/kg) increased with increasing days of food deprivation. A more detailed analysis of the results indicated that both the peak effect and the duration of drug action were significantly increased by food deprivation.[6]

During starvation liver metabolism of certain psychoactive drugs is altered.[11,23] ANGEL,[1] for example, gave intraperitoneal injections of cocaine and found higher levels of the drug in the brains of starved rats as compared with controls. These findings suggest that the increased stimulant effects of amphetamine observed during food deprivation may depend on impaired metabolism of the drug. To test this hypothesis H^3-amphetamine was injected intraperitoneally (i.p.) into animals food-deprived for 4 days and controls. One hour later, when the peak behavioural effects occurred, the rats were sacrificed and the whole brain levels of H^3-amphetamine and its metabolites were measured. No significant difference between control and test groups were obtained, indicating that impaired metabolism of amphetamine cannot account for its increased psychomotor stimulant effects during food deprivation (Table 1).

Food deprivation is a highly stressful situation for the rat, and when stressed, increased turnover of brain norepinephrine (NE) has been reported by a number of investigators. For example, foot shock,[2,40] extremes in temperature,[8,36] immobilisation,[9,46] and extreme muscular exercise[22] have all been shown to increase the turnover of brain NE. Dopamine (DA) turnover can also be altered by stress.[2,3,28] The increased responsiveness to amphetamine during food deprivation may therefore reflect changes in the interaction between amphetamine and brain CA turnover.

TABLE 1. EFFECT OF FOOD DEPRIVATION ON WHOLE BRAIN LEVELS OF *d*-AMPHETAMINE

	Whole supernatant ($\times 10^4$ dis/min per g)	Extracted amphetamine ($\times 10^4$ dis/min per g)
2 days food deprivation		
Control	3·90 ± 0·32	3·79 ± 0·57
Food deprived	3·29 ± 0·25	2·85 ± 0·25
4 days food deprivation		
Control	3·39 ± 0·19	3·26 ± 0·25
Food deprived	3·21 ± 0·30	3·17 ± 0·30

Rats were injected intraperitoneally with a solution of unlabelled *d*-amphetamine sulfate (1·0 mg/kg) containing ^{3}H-*d*-amphetamine sulfate ($8{\cdot}56 \times 10^7$ dis/min per g.). Animals were sacrificed 1 hr later and the whole brain (including cerebellum) was homogenised in acid. An aliquot of the supernatant was counted (whole supernatant) and amphetamine was extracted from the remaining supernatant by the method of GLOWINSKI, AXELROD and IVERSEN.[20] Data represent means (± S.E.M.) of 4 animals in each group. No statistically significant differences were obtained between the control and food deprived groups.

The fact that stress significantly increases amphetamine toxicity is consistent with such a hypothesis.[21,44] To examine this possibility, animals were maintained on *ad libitum* food and water or they were food deprived for 4 days and then injected i.p. with α-methyl-*p*-tyrosine (α-MPT). Food deprivation did not significantly alter the rate of depletion of whole brain NE or DA after α-MPT (Table 2). These results are consistent with the observation that 4 days of food deprivation do not alter tyrosine hydroxylase [EC.1.14.3a] activity in the mid-brain, hypothalamus, hippocampus or caudate (FIBIGER and MCGEER, unpublished). It appears unlikely therefore that the increased stimulant effects of amphetamine during food deprivation are the result of an altered interaction between amphetamine and CA turnover, and at present the neurochemical basis of this response remains to be elucidated.

TABLE 2. EFFECT OF FOOD DEPRIVATION (4 DAYS) ON α-METHYL-*p*-TYROSINE INDUCED DEPLETION OF BRAIN NORADRENALINE AND DOPAMINE

	0 hr	0·5 hr	2·0 hr
Noradrenaline (μg/g)			
Controls	0·338 ± 0·002 (4)	0·249 ± 0·015 (9)	0·137 ± 0·019 (6)
Food deprived	0·377 ± 0·015 (4)	0·226 ± 0·015 (9)	0·147 ± 0·018 (6)
Dopamine (μg/g)			
Controls	0·466 ± 0·036 (4)	0·415 ± 0·009 (9)	0·259 ± 0·014 (8)
Food deprived	0·495 ± 0·028 (4)	0·427 ± 0·017 (9)	0·260 ± 0·009 (9)

Rats were food deprived for 4 days or maintained on *ad libitum* food and water. L-α-methyl-*p*-tyrosine (Regis Co.) was injected intraperitoneally (200 mg/kg) and the animals were sacrificed at various intervals thereafter. The whole brain (including cerebellum) was homogenised in acid and brain noradrenaline and dopamine levels were determined fluorometrically by the method of MCGEER and MCGEER.[29] Numbers in parentheses indicate number of animals in each group. Data represent means (± S.E.M.). No statistically significant differences were obtained between the control and food deprived groups.

There are several possibilities which require further investigation however. In the above experiments only the whole brain levels of amphetamine and its metabolites were measured. Food deprivation may produce changes in either the regional accumulation of amphetamine in brain or in the subcellular distribution of the drug. Similarly, although whole brain DA and NE turnover do not appear to be affected by

food deprivation, discrete and local changes may occur in the turnover of these amines which are obscured by whole brain measurements. Strong support for the latter possibility has recently been provided by FRIEDMAN, STARR and GERSHON[19] who found that 22 hr of food deprivation significantly increased α-MPT induced depletion of NE in the hypothalamus but not in the remainder of the brain. These workers also observed that whole brain DA depletion was slightly increased in food-deprived rats 4 hrs after α-MPT, but that finding was not supported by the present experiments.

Whatever mechanisms underlie the increased amphetamine response during food deprivation, it is obvious that the nutritive state of the animal can be of considerable importance in determining the magnitude and the nature of the drug response. An example of the difficulties which can be encountered when this factor is not controlled for is found in the reports of increased sensitivity to amphetamine and increased spontaneous locomotor activity after chronic reserpine treatment in the rat.[33,34,37] Such treatment produces *subsensitivity* to indirectly acting sympathomimetics such as amphetamine in the peripheral nervous system.[41] The resolution of this inconsistency may lie in the fact that when rats are treated chronically (10–14 days) with small doses of reserpine, many of the animals become hypophagic and hypodipsic and show considerable losses in body weight.[15,37] The importance of this factor in the potentiation of amphetamine stimulation after chronic reserpine treatment has been evaluated.[15] In accordance with the previous reports, groups of rats given chronic reserpine treatment showed significantly increased spontaneous locomotor activity and an enhanced response to D-amphetamine as compared with controls. It is noteworthy however, that the chronic reserpine treatment produced variable effects on final body weight and the increased spontaneous activity and the enhanced responsiveness to amphetamine were observed only in those individual animals which suffered marked weight loss. In a second experiment, the food intake of the control group was restricted so that it was similar to the *ad libitum* intake of the rats treated with reserpine. In this case, where both the saline and the reserpine-treated groups suffered similar weight losses over 10 days, the chronic reserpine group was in no sense more responsive to amphetamine than were the controls. On the contrary, on one measure (lowest amphetamine dosage which significantly increased activity) the control group showed slightly greater responsiveness to amphetamine than did the chronic reserpine group. Taken together, these experiments suggest that the increased stimulant effect of D-amphetamine and the increase in spontaneous activity which develops during chronic reserpine administration is a result of the severe hypophagia observed during the drug treatment.[15] These results again point to the importance of controlling for the nutritive state of the organism in behavioural pharmacological research.

The fact that chronic reserpine administration produces hypophagia and hypodipsia supports the view that brain biogenic amines may be of critical importance in regulating food and water intake in the rat.[24,31] This hypothesis has recently received strong experimental support in the finding that 6-hydroxydopamine (6-OHDA), an agent which can selectively destroy CA neurons in the brain, can when injected intraventricularly into monoamine oxidase (MAO) inhibited animals or directly into the substantia nigra, produce profound aphagia and adipsia.[16,17,43] The syndrome produced shows a remarkable resemblance to the well-known "lateral hypothalamic syndrome" in which animals with bilateral electrolytic lesions of the lateral hypothalamus gradually recover from aphagia and adipsia but continue to show more

subtle but permanent deficits.[17,39,47] These more permanent deficits are also found in the 6-OHDA treated animals.[17,47] To date, these experiments suggest that the DA nigrostriatal projection may have a major role in the control of food and water intake,[17,43] since bilateral injections of 6-OHDA into the substantia nigra produce a syndrome which most closely resembles the lateral hypothalamic syndrome. A contribution of the mesolimbic system, the other major DA projection,[42] to this syndrome cannot at present be ruled out however. MALER and FIBIGER (unpublished observations) using the Fink Heimer technique, have found that when 6-OHDA is injected into the substantia nigra in the doses necessary to destroy the nigro-striatal projection (8 μg), extensive damage is also observed in the mesolimbic DA system.

TABLE 3. EFFECTS OF 6-HYDROXYDOPAMINE ON LOCOMOTOR STIMULATION, STEREOTYPY AND ANOREXIA INDUCED BY AMPHETAMINE SULFATE

	Locomotor stimulation	Stereotypy	Anorexia	
			Baseline intake	Amphetamine intake
Amphetamine dosage (expressed as the salt)	1 mg/kg	5 mg/kg		1·5 mg/kg
Controls	491 ± 72	3·20 ± 0·10	9·07 ± 0·77	1·97 ± 0·30
6-OHDA	34 ± 10*	0·59 ± 0·14*	8·24 ± 0·38	6·75 ± 0·54*

6-hydroxydopamine treated animals received intraventricular injections (250 μg) into the lateral ventricle 30 min after tranylcypromine sulfate (5 mg/kg i.p.). Controls received intraventricular injections of the vehicle. Behavioural tests were conducted after recovery of food and water intake. In the locomotor stimulation test, animals were adapted to a photocell cage for 1 hr, then injected i.p. with *d*-amphetamine and the resulting activity (number of photobeams interrupted) was recorded for 1 hr. In the stereotypy test, stereotypy was measured by the method of FIBIGER, FIBIGER and ZIS[42] for 3 hr after i.p. amphetamine administration. In the anorexia test food intake (in g) during 75 min (after 24 hr of food deprivation) was measured after saline (baseline) or amphetamine injections. Data represent means (± S.E.M.) of 10 animals in each group. *Significantly different from controls. $P < 0{\cdot}01$.

The use of 6-OHDA has also permitted further investigations of the neurochemical mechanisms underlying the behavioural effects of amphetamine. Because considerable evidence indicates that amphetamine is an indirectly acting sympathomimetic,[25,38,45] it is somewhat surprising that large doses of 6-OHDA have failed to attenuate amphetamine-induced motor-stimulation despite a reduction of brain catecholamines by 75–80 per cent.[12] This finding is in accord with other reports which have described a general lack of long-lasting behavioural changes (except for increased irritability) after intraventricular 6-OHDA treatment.[5,27,32,35] In these reports 6-OHDA was administered to animals in which MAO had not been inhibited, and the depletion of brain DA was consequently less complete than that of brain NE. Since MAO inhibition increases the destructive capacity of 6-OHDA, particularly on DA neurons,[4,16] some of these earlier experiments were repeated in animals in which MAO was inhibited before the intraventricular injection of 6-OHDA.[18] This procedure produced a 90 per cent depletion in both brain NE and brain DA. After the animals had recovered the ability to regulate food and water intake, they were tested for amphetamine-induced motor stimulation, stereotyped behaviour, and amphetamine anorexia. In these animals all of the above behavioural effects of amphetamine were drastically reduced[17,18] (Table 3), suggesting that the earlier failures to significantly alter the behavioural effects of amphetamine by 6-OHDA treatment were due to an incomplete destruction of CA neurons.

These observations are consistent with the view that amphetamine exerts its behavioural effects indirectly through its action on brain catecholamines, but suggest that the CA systems which subserve these behaviours are present in large excess of the requirements for the maintenance of normal behavioural function. Since only slight effects on behaviour are observed when 70–80 per cent of CA systems are destroyed by 6-OHDA and marked effects are sometimes not observed until this destruction reaches 90 per cent or more, the relationship between brain CA levels and behaviour is not linear.

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