TOXICITY OF AMPHETAMINE-RELATED DRUGS AND RESULTING BEHAVIORAL CHANGES

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ABSTRACT. It is well known that brief exposure to a variety of amphetamine analogs causes long-lasting neurotoxic changes in dopamine and/or serotonin neurons in the CNS. The functional consequences of this toxicity have not been determined. Administration of fenfluramine to rats twice daily for 4 days produces long-lasting depletions of serotonin and results in tolerance to both anorectic and neurochemical effects of acute fenfluramine. On the other hand, a 4 day regimen of MDMA enhances the effects of acute MDMA on a DRL schedule of reinforcement as well as morphine analgesia. These studies indicate that short-term exposure to neurotoxic amphetamines can cause long-lasting changes in sensitivity to either the same or different drugs.

Introduction

Certain substituted amphetamines are toxic to dopamine (DA) and/or serotonin (5-HT) neurons in the CNS, and we will review here some neurochemical, behavioral, and pharmacological data relevant to potential long-lasting effects. Due to their abuse potential, the question arose as to whether amphetamine-like drugs may have long-lasting and/or toxic effects on the CNS. In very high doses such as those used by human amphetamine abusers, the amphetamine-type drugs can lead to a psychotic state which has paranoid delusional symptoms that are very often indistinguishable from an acute psychotic episode seen in patients with schizophrenia [10]. Although the symptoms produced in humans suggested that amphetamine-type of drugs may engender a neurotoxic response in the CNS, there was no experimental evidence of brain pathology until the early 1970s. Koda and Gibb [12] reported that 18 hrs after a dosing regimen in which methamphetamine (MA) was injected every 5 hrs, the Vmax for tyrosine hydroxylase was reduced. Seiden et al. [19] reported that in monkeys administered amphetamine for several months but sacrificed 3 to 6 months after the last injection, the levels of DA in the brain were reduced. This long-term reduction in brain DA suggested that MA was toxic to DA cells.

The original observation of long-term depletions of DA was made during a study of the development of tolerance to the effects of daily injections of MA in rhesus monkeys [5]. Behavioral tolerance to MA's effects on a DRL task persisted long after the repeated MA regimen. Monkeys treated with repeated MA also showed reduced sensitivity to apomorphine and increased sensitivity to haloperidol [4]. Increased sensitivity to haloperidol and tolerance to MA's effects on locomotor activity of rats [15] and a force-lever task in rhesus monkeys [1] have also been reported to occur after a regimen of MA. Since in each of these studies repeated administration of MA resulted in substantial DA depletions, tolerance to subsequent MA injections is most likely related to selective destruction of DA terminals.

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Neurotoxicity can be established by three criteria: 1) long-lasting decreases in levels of transmitter and activity of enzyme(s) that are rate limiting for the transmitter; 2) a reduction in the number of reuptake sites for the transmitter, and 3) neuronal degeneration in areas of the brain specific to the transmitter. It is important to stress that these criteria must be met before neurotoxicity can be established. Neurotoxicity has been established for MA, 3,4-methylenedioxyamphetamine (MDA), and 3,4-methylenedioxymethylamphetamine (MDMA), see [20]. These drugs deplete levels of 5-HT, reduce numbers of 5-HT uptake sites , depress tryptophan hydroxylase activity and produce signs of neuronal degeneration [20]. Unlike MA which also has effects on DA, neurotoxicity of MDA and MDMA is directed primarily toward the 5-HT system since much higher doses are required to produce significant reductions in the levels of DA or metabolites. Fenfluramine is a ring-substituted amphetamine derivative which also meets the criteria for neurotoxicity. Depletions of 5-HT lasting up to 6 months after cessation of drug treatment have been reported [3,6,7,8,11,18,21]. Other long-lasting effects of fenfluramine include a decrease in 5-HT uptake sites [18] and tryptophan hydroxylase activity [21]. Although once thought to be equivocal, fenfluramine has been shown to produce signs of neuronal degeneration and recent immunohistochemical studies support the idea that fenfluramine produces morphological damage to 5-HT terminal fields [2,6,7,8]. Together, the neurochemical and histological data indicate that these substituted amphetamines are neurotoxic.

We have recently conducted several studies to address the question of long-term consequences of 5-HT neurotoxicity produced by fenfluramine and MDMA. Changes in sensitivity to the effects of these drugs on anorexia [11] and operant behavior [14] were examined. Additionally, we have recently reported changes in sensitivity to morphine resulting from a brief regimen of MDMA [16]. Each of the studies reviewed here utilized pharmacological techniques to unmask potential behavioral deficits produced by the neurotoxic regimen of drug.

Methods

General procedure. Male Sprague-Dawley rats (Harlan, IN), weighing 200 - 250 g at the start of each experiment, were housed individually in stainless steel cages. Food (Teklad Rat and Mouse Diet, Teklad, Winfield, IA) and water were continuously available. Room lights were on a 12 hour dark-light cycle (lights on from 0700-1900) and room temperature was maintained at approximately 24 °C. To confirm the extent of monoamine depletions at various times following a regimen of repeated drug, rats were sacrificed by guillotine and their brains were dissected over ice into regions comprising somatosensory cortex, striatum, hippocampus, and hypothalamus [9]. Brain sections were wrapped in aluminum foil, stored in liquid nitrogen, and assayed for DA, norepinephrine (NE), and 5-HT by use of HPLC-EC[13]. In each of the studies, neither NE nor DA levels were significantly different from control in any of the brain regions analyzed.

Anorexia. Rats previously allowed to drink sweetened condensed milk during daily 15 min sessions were treated with fenfluramine (6.25 mg/kg twice daily for 4 days) or saline. Two or 8 weeks after the last daily fenfluramine injection, rats were administered fenfluramine acutely, tested for milk intake, and sacrificed 2 hours later.

DRL 72 sec behavior. The behavioral effects of single injections of MDMA on rats maintained under a differential reinforcement-of-low rate 72-second schedule (DRL 72-sec; see [17]) were compared before and after repeated administration of MDMA (6 mg/kg twice daily for 4 days).

Morphine Analgesia. Rats were injected twice a day for 4 days with 20 mg/kg MDMA or saline, and, after 2 weeks, nociception was determined by measuring reaction time to the tail immersion in heated water (55° C). After determining baseline reaction times, rats were randomly assigned to 4 groups receiving saline or morphine (2.5, 3.55, or 5 mg/kg, sc), and the nociceptive test was repeated at various times after drug or saline administration. For further details see [16].

Results

Anorexia. Acute administration of fenfluramine produced a dose-related decrease in milk intake and 5-HT levels in various brain regions. The 4 day regimen of fenfluramine resulted in tolerance to the anorectic effect of acute fenfluramine. Levels of 5-HT were depleted 2 and to a lesser extent, 8 weeks after the fenfluramine regimen and there was apparent tolerance to the acute 5-HT-depleting effect of fenfluramine as a result of the 4 day fenfluramine regimen. Partial recovery of both behavioral and neurochemical tolerance to fenfluramine was observed 8 weeks after the 4 day regimen.

DRL 72-sec behavior. Acute administration of MDMA either decreased reinforcement rate (1-6 mg/kg) or increased response rate (4 mg/kg) of rats performing on the DRL 72-sec schedule. This effect is typical of amphetamines and other psychomotor stimulants. Four weeks after repeated administration of MDMA there was an increase in sensitivity to the effects of MDMA. Doses of 2, 4 and 6 mg/kg of MDMA resulted in increases in response rate that were significantly greater after repeated MDMA administration than before. Doses of 0.5, 2, and 6 mg/kg of MDMA resulted in decreases of reinforcement rate that were significantly greater after repeated MDMA administration than before. Repeated administration of MDMA resulted in long term depletion of serotonin levels by 30 - 50 % in the amygdala, neostriatum, hippocampus and the frontal cortex.

Morphine analgesia. The analgesic effects of morphine were assessed in rats previously treated with a regimen of MDMA which significantly decreased 5-HT levels in all of the brain regions examined. Morphine administration produced an analgesic effect that was stronger and more prolonged in MDMA- than in saline-pretreated rats, indicating that morphine was more potent as a consequence of a neurotoxic regimen of MDMA.

Discussion

It is clear that a neurotoxic regimen of amphetamine-related drugs produces either tolerance or sensitization to the effects of subsequent drug treatment. Repeated administration of fenfluramine produced decreases in 5-HT and tolerance to the anorectic effects of fenfluramine [11]. The recovery of sensitivity to acute injections of fenfluramine was related to the level of 5-HT depletion, indicating that tolerance to fenfluramine anorexia was due to the long-lasting effects on 5-HT. In contrast to studies in which tolerance to MA was observed, sensitivity to the effects of MDMA on DRL performance in rats was found to increase as a consequence of a neurotoxic regimen of MDMA [14]. Since levels of 5-HT but not NE or DA were significantly depleted following the MDMA regimen, the behavioral results suggest that 5-HT neurons normally exert an inhibitory action upon the psychomotor stimulant effects of MDMA. Since the psychomotor stimulant effects of amphetamines appear to be mediated primarily by the DA system, these results provide evidence that 5-HT and DA may represent opposing systems insofar as they play a role in DRL schedule-controlled behavior. Finally, a neurotoxic regimen of MDMA enhanced morphine analgesia [16].

It should be noted that persisting behavioral effects of the chronic regimen of drug, in the absence of such pharmacological challenges have not, to our knowledge, been reported. Baseline changes of behavior were not found in any of the studies presented here or previously reported [1,4,5,15]. While pharmacological probes reveal an underlying change in DA and/or 5-HT function, the nature of behavioral deficits in the absence of drug challenge remains to be determined.

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