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BRIEF COMMUNICATION

Effects of Anisomycin and CNS Stimulants on Brain Catecholamine Synthesis[1]

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LUNDGREN, P. AND L. A. CARR. *Effects of anisomycin and CNS stimulants on brain catecholamine synthesis.* PHARMAC. BIOCHEM. BEHAV. 9(4) 559–561, 1978.—Mice were injected with anisomycin, an inhibitor of cerebral protein synthesis; d-amphetamine, strychnine, or caffeine was administered 30 min later. Fifteen min before sacrifice at 1 or 2 hr after injection of anisomycin, ^{3}H-tyrosine was injected intravenously, and catecholamine synthesis rates were estimated by measurement of the specific activity of ^{3}H-tyrosine and the accumulation of ^{3}H-norepinephrine and ^{3}H-dopamine. Anisomycin decreased synthesis rates of catecholamines, but this effect was not significantly affected by any of the CNS stimulants. These results suggest that the recently reported reversal of anisomycin-induced amnesia by these stimulants is not due to an attenuation of brain catecholamine synthesis inhibition.

Anisomycin Memory Catecholamines CNS stimulants

IN STUDIES attempting to identify the neurochemical systems involved in storage and retrieval of memory, much attention has been focused on the effects of inhibitors of protein synthesis, particularly cycloheximide, acetoxycycloheximide, and anisomycin (ANI). It has been shown that these drugs produce amnesia for a specific learning task when given shortly before or after training, and it is generally believed that this action is due to inhibition of cerebral protein synthesis. However, it has recently become apparent that these drugs have effects on central catecholamine-containing neurons which may account for the disruption of long-term memory. For example, inhibitors of protein synthesis have been found to inhibit tyrosine hydroxylase [3] and to inhibit catecholamine synthesis [2,6]. Furthermore, the amnesic effect of these agents may be prevented by drugs which would be expected to enhance catecholamine neuron function [8, 9, 10, 13]. Although these studies clearly show that treatments affecting catecholamine neuron function may prevent the amnesic effect of protein synthesis inhibitors, this may not necessarily be a conclusive argument that impairment of adrenergic function is the primary effect responsible for the amnesia produced by these drugs, since similar effects have been reported after administration of CNS stimulants which may not act solely on catecholamine neurons. Flood and co-workers [5] have shown that strychnine, caffeine, nicotine, picrotoxin, and d-amphetamine protect against amnesia induced by ANI, whereas chloral hydrate and sodium phenobarbital enhanced the amnesia. Since these treatments would not be expected to affect any single neurotransmitter system in particular, it was concluded that modulation of CNS arousal by these drugs is the mechanism by which they affect memory.

The purpose of the experiments reported here was to study the effects of anisomycin on catecholamine synthesis and to determine whether these effects are modified by a number of CNS stimulants.

METHODS

Animals used were male C57/BL6 mice (Jackson Labs) between 9 and 14 weeks old and weighing 18–29 g. They were housed in small groups of 5–6 animals and provided with water and Purina Rat Chow ad lib. All drugs were dissolved in 0.14 M NaCl buffered to pH 7 with 0.01 M sodium phosphate. Anisomycin (2-p-methoxyphenyl-3-acetoxy-4-hydroxypyrrolidine) was a gift from Dr. Nathan Belcher of Pfizer Pharmaceuticals and was injected subcutaneously (2[illegible] mg/kg) on the back of the neck. Thirty min after ANI was administered, saline, strychnine sulfate (0.3 mg/kg, Merck), caffeine (anhydrous, 100 mg/kg, Sigma Chemical Co.), or d-amphetamine sulfate (5 mg/kg, Smith, Kline, and French, Inc.) was injected intraperitoneally. The injection volume for all drugs was 0.01 ml/g body weight. ^{3}H-tyrosine (3,5-^{3}H-tyrosine, specific activity 40-60 Ci/mmole, New England Nuclear) was evaporated to dryness under nitrogen and re-

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TABLE 1

EFFECTS OF ANISOMYCIN AND STIMULANTS ON ENDOGENOUS TYROSINE CONCENTRATION

	1 hour	2 hours
Saline	33.1 ± 0.8(4)*	28.4 ± 1.7(4)
ANI + Saline	54.7 ± 1.9(4)†	50.6 ± 2.1(4)†
ANI + D-AMP	54.4 ± 4.1(4)†	50.8 ± 3.5(5)†
ANI + CAFF	56.2 ± 1.1(4)†	47.6 ± 1.4(4)†
ANI + STR	48.5 ± 4.5(4)†	60.6 ± 8.2(4)†

*μg tyrosine/g brain tissue ± SEM. Number of animals is shown in parentheses. Stimulants were injected 30 min after ANI, and animals were sacrificed at 1 or 2 hr after ANI. Drug doses were as follows: ANI–25 mg/kg, D-AMP–5 mg/kg, STR–0.3 mg/kg, CAFF–100 mg/kg.

† Significantly greater than control $p < 0.01$

TABLE 2

EFFECTS OF ANISOMYCIN AND STIMULANTS ON NOREPINEPHRINE SYNTHESIS

	1 hour	2 hours
Saline	0.030 ± 0.005(4)*	0.034 ± 0.007(4)
ANI + Saline	0.017 ± 0.003(4)†	0.012 ± 0.002(4)‡
ANI + D-AMP	0.010 ± 0.001(4)‡	0.006 ± 0.001(5)‡
ANI + CAFF	0.014 ± 0.002(4)‡	0.011 ± 0.001(4)‡
ANI + STR	0.018 ± 0.004(4)†	0.010 ± 0.002(4)‡

Refer to legend of Table 1 for details.

* μg/g brain tissue/15 min ± SEM.

† Significantly decreased from control $p < 0.05$

‡ Significantly decreased from control $p < 0.01$

dissolved in phosphate buffered saline to a concentration of 500 μCi/ml. Each mouse received 0.2 ml (100 μCi) of this solution intravenously via the lateral tail vein either 45 or 105 min after the injection of ANI and sacrificed 15 min later by cervical dislocation.

Immediately after sacrifice, each mouse was decapitated, the brain was removed, and the cerebellum, olfactory bulbs, and lower brain stem were dissected off. The remaining tissue was weighed and homogenized in 3 ml of ice-cold 0.4 N $HClO_4$, and the homogenate was centrifuged for 5 min at 10,000 X g. The supernatant was then decanted and the pellet rehomogenized and centrifuged as before, and the 2 supernatants were combined and frozen until assayed.

Endogenous tyrosine was measured fluorometrically by the method of Waalkes and Udenfriend [17]. The concentrations of ^{3}H-tyrosine, ^{3}H-norepinephrine, and ^{3}H-dopamine were measured in the brain homogenates by methods previously reported [16]. The rates of catecholamine synthesis were calculated by dividing the brain concentration of ^{3}H-catecholamine by the specific activity of tyrosine [20].

Data were analyzed by a one-way ANOVA across each time level, and where comparison of individual means to one another was desired, this was done by the Duncan Studentized range test [19].

RESULTS

Administration of ANI alone increased the brain concentration of endogenous tyrosine at 1 and 2 hr (Table 1). The addition of stimulants did not alter the effect of ANI on tyrosine. The specific activity of ^{3}H-tyrosine was increased by all drug treatments at 2 hr due to a greater relative increase in ^{3}H-tyrosine concentration than in endogenous levels of tyrosine.

TABLE 3

EFFECTS OF ANISOMYCIN AND STIMULANTS ON DOPAMINE SYNTHESIS

	1 hour	2 hours
Saline	0.026 ± 0.004(4)*	0.040 ± 0.007(4)
ANI + Saline	0.012 ± 0.002(4)†	0.013 ± 0.002(4)†
ANI + D-AMP	0.010 ± 0.002(4)‡	0.011 ± 0.001(4)†
ANI + CAFF	0.013 ± 0.003(4)†	0.013 ± 0.002(4)†
ANI + STR	0.012 ± 0.002(4)†	0.013 ± 0.002(4)†

Refer to legend of Table 1 for details.

* μg/g brain tissue/15 min ± SEM.

† Significantly decreased from control $p < 0.01$

ANI decreased the synthesis rates of norepinephrine and dopamine at both time intervals studied (Tables 2 and 3). ANI and d-amphetamine seemed to have a greater effect on norepinephrine synthesis than ANI alone, but statistical analysis of these 2 treatments did not reveal a significant difference. None of the stimulants used in this study significantly modified the inhibition of catecholamine synthesis by ANI.

DISCUSSION

The effects of ANI on endogenous tyrosine levels in the brain are in agreement with other studies [2] and are probably due to a reduction of tyrosine incorporation into protein. In preliminary studies, it was found that the dose of ANI used in the present report inhibited protein synthesis by 90% at ½ hr, 89% at 1 hr, and 85% at 2 hr. Spanis and Squire [14] have shown that the increase in endogenous tyrosine levels alone cannot account for the amnesia resulting from administration of protein synthesis inhibitors. It is also apparent from the present results that the reported protective effects of CNS stimulants on amnesia induced by ANI [4,5] are not due to an alteration of endogenous tyrosine levels.

ANI inhibited catecholamine synthesis for at least 2 hr, as was also reported by Flexner and Goodman [2]. Considering the reports implicating brain catecholamine neuron systems in memory formation [1, 8, 11], it is likely that inhibition of catecholamine synthesis may be at least partly responsible for the amnesia caused by ANI. A contrasting view has been proposed by Squire *et al.*, [15], who reported that a dose of a-methyl-p-tyrosine (AMPT) that inhibited tyrosine hydroxylase to the same degree as ANI had no amnesic effect. Since these authors felt that it was unlikely that inhibition of catecholamine synthesis by ANI was responsible for its amnesic effect, this action was attributed to inhibition of protein synthesis. However, other studies argue against this mechanism since drugs which prevent or attenuate the amnesia do not alter the degree of protein synthesis inhibition [5]. It is also possible that the inhibition of catecholamine synthesis by ANI is an indirect result of an action on some other dynamic aspect of catecholamine neuron function. For example, if ANI interferes with neuronal release of

catecholamines, as suggested by Flexner and Goodman [2], then reduction of catecholamine synthesis rates by ANI may be due to feedback inhibition of tyrosine hydroxylase by increased intraneuronal levels of catecholamines [18]. If this is the case, then the effects of AMPT on catecholamine neuron systems would not be analogous to those of ANI, so the conclusion reached by Squire *et al.*, may be in doubt. This proposed feedback mechanism is also supported by the observation in the present study that the inhibition of catecholamine synthesis by ANI seems to be potentiated by d-amphetamine. This dose of d-amphetamine has been reported to inhibit catecholamine synthesis [12], presumably due to feedback inhibition of tyrosine hydroxylase as a result of decreased intraneuronal binding of catecholamines. If ANI causes a decreased release of catecholamines, then the inhibition of catecholamine synthesis after ANI may be potentiated after d-amphetamine by a further increase in intraneuronal catecholamine levels.

The stimulants used in this study did not prevent the inhibition of catecholamine synthesis by ANI. Therefore, reversal of ANI-induced amnesia by these drugs is apparently not due to a direct effect on catecholamine synthesis. However, this finding does not necessarily weaken the hypothesis that production of amnesia by ANI is due, in part, to effects on catecholamine neuron systems. It is possible that the stimulants used in this study compensate for impairment of adrenergic function by actions on other neuronal systems. Flood *et al.*, [4] have presented evidence that restoration of normal memory function by administration of stimulants after ANI is due to increased CNS arousal. In normal animals, catecholamine neuron systems may modulate levels of arousal as part of the memory storage process [7], so perhaps the general CNS stimulants found to prevent amnesia compensate for lowered levels of arousal caused by impairment of adrenergic systems by inhibitors of protein synthesis.

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