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July 19, 1967

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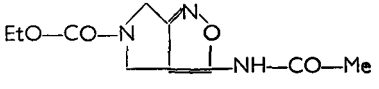
Effects of ethyl 3-acetamido-4*H*-pyrrolo[3, 4-c]-isoxazole-5(6*H*)carboxylate on tissue levels of catecholamines and 5-hydroxytryptamine in the rat

SIR,—Various agents have been found which cause a marked lowering of the tissue levels of catecholamines or 5-hydroxytryptamine (5-HT) or both. Some of these depletors, such as α-methyldopa, guanethidine or reserpine, are used in hypertension. The compound, ethyl 3-acetamido-4*H*-pyrrolo-[3,4-*c*]isoxazole-5(6*H*) carboxylate (I;CL-62375), has recently been found to cause hypotension in the rat. We now report that administration of this compound to the rat causes alterations in the tissue levels of catecholamines and 5-HT.



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Brain catecholamine levels (Lippmann & Wishnick, 1965), brain 5-HT (Bogdanski, Pletscher & others, 1956), heart noradrenaline (Anton & Sayre, 1962) and adrenal catecholamines (Lippmann & Wishnick, 1965), were measured in female rats, Sherman strain, of about 150 g.

CL-62375 was administered intraperitoneally in a single injection (0·5 ml 1% starch, M/15 potassium phosphate buffer, pH 7·0) at 100, 150, 250 or 400 mg/kg and the animals were decapitated 5 hr later. In the heart there was a decline in the noradrenaline level of 70, 42 and 35% at 250, 150 and 100 mg/kg, respectively. In the brain there was a lowering in the catecholamine content of 70% at 250 mg/kg and 40% at 150 mg/kg. The brain 5-HT showed a maximum decline of 30% at the 250 mg/kg level. The 400 mg/kg level was lethal. Thus, there was an appreciable effect on the catecholamine levels in the heart and brain

whereas there was only a slight effect on the 5-HT in the brain. The animals were not sedated and showed only a slight ptosis.

A dose of 50 mg/kg of CL-62375 was administered intraperitoneally three times at 3 hrly intervals and the animals were killed 2 hr after the last injection. A decline of 59% in the noradrenaline level of the heart (μg/g $\pm$ s.e.: control 1·07 $\pm$ 0·06; treated 0·44 $\pm$ 0·04, $P < 0·001$) and a 33% decline in the catecholamine content (μg/g $\pm$ s.e.: control 0·33 $\pm$ 0·03; treated 0·22 $\pm$ 0·006, $P < 0·05$) of the brain were observed. There was a 59% lowering of the brain 5-HT (μg/g $\pm$ s.e.: control 0·83 $\pm$ 0·16; treated 0·34 $\pm$ 0·02, $P < 0·001$). A 31% drop in the catecholamine level of the adrenals also occurred (μg/pair $\pm$ s.e.: control 22·22 $\pm$ 0·96; treated 15·33 $\pm$ 0·27, $P < 0·001$). After the first treatment the animals exhibited a slight ptosis and were not sedated; subsequent treatments did not cause sedation.

To determine the duration of the effects of repeated administration of CL-62375, the animals received 3 injections (50 mg/kg, i.p.) at 3 hrly intervals. The level of noradrenaline in the heart declined 59, 67 and 47% at 2, 8 and 18 hr, respectively, after the last treatment. Thus under these conditions a maximum depletion was observed at 8 hr and the levels were still appreciably lowered after 18 hr. In the brain, the catecholamine levels dropped 33% at 2 hr after the last dose and no significant reduction was observed after 8 or 18 hr. The brain 5-HT was lowered 59, 67 and 40% after 2, 8 and 18 hr.

A decline in the endogenous biogenic amine levels may arise from an alteration in the storage mechanisms; i.e., uptake and release, or an alteration in the synthesis of the amines. The effects of CL-62375 can be compared with compounds exhibiting these activities; i.e., α-methyl-*m*-tyrosine, a releaser, and α-methyltyrosine, a synthesis inhibitor. After a single administration CL-62375, α-methyl-*m*-tyrosine (Hess, Connamacher & others, 1961; Weissmann & Koe, 1965) and α-methyltyrosine (Spector, Sjoerdsma & Udenfriend, 1965) are similar in that they cause an appreciable depletion of both heart and brain catecholamines and cause only a small or no decrease in 5-HT levels. After repeated administration CL-62375 exhibits a decline in catecholamines similar to that produced by α-methyltyrosine (Spector & others, 1965); in contrast, CL-62375 causes a large decrease in the brain 5-HT whereas α-methyltyrosine has no effect (Spector & others, 1965). The noradrenaline-depleting action of CL-62375 might thus be the basis for its hypotensive activity.

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August 8, 1967

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