Short communications

The effects of an anti-atherosclerotic drug, pyridinolcarbamate, on heart, vascular and brain biogenic amines

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The anti-atherosclerotic drug pyridinolcarbamate was investigated in the rat for its effects on heart, blood vessel and brain biogenic amines. The drug increased the concentration of noradrenaline in all tissues and of noradrenaline and 5-hydroxytryptamine in the brain. The possibility of a link between biogenic amines and atherosclerotic disease is discussed.

Peripheral arterial diseases are treated by a variety of procedures including surgery, sympathectomy and drugs causing vasodilatation (Owens, 1972, Richards, 1970). Atherosclerosis is a frequent contributing factor in vascular disease and drugs which modify or prevent atheromatous vascular lesions by affecting cholesterol metabolism and disposition are currently being used and evaluated (Kritchevsky, 1971). We were interested in investigating the effect of atherosclerosis on cardiovascular amine disposition. During the course of these studies we observed that a new anti-atherosclerotic drug, pyridinolcarbamate (2, 6-bis (hydroxymethyl) pyridine) bis (*N*-methylcarbamate), itself produced alterations in tissue catecholamine and 5-hydroxytryptamine content in the rat. The present studies indicate that pyridinolcarbamate may affect the cardiovascular system directly by modifying the noradrenaline content of heart and vascular tissue and possibly, indirectly, by raising the concentration of brain noradrenaline and 5-hydroxytryptamine.

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Methods.—Female Sprague Dawley rats weighing 200 grammes were used. Rats were killed by cervical dislocation and tissues rapidly dissected out, frozen on dry ice and stored frozen until analysed. Vascular tissues were pooled and fluorometrically assayed for noradrenaline as previously described (Berkowitz, Tarver & Spector, 1971 ; Berkowitz, Spector & Tarver, 1972). Heart and brain were assayed individually for noradrenaline (Anton & Sayre, 1962) or 5 hydroxytryptamine (Snyder, Axelrod & Zweig, 1965). Monoamine oxidase activity was estimated by the method of Weissbach, Smith, Daly, Witkop & Udenfriend (1960). The method of Udenfriend, Weissbach & Brodie (1958) was used to estimate brain 5-hydroxyindole acetic acid. Cardiovascular histamine content was measured as described by Howland & Spector (1972).

Results.—Administration of single daily doses of pyridinolcarbamate 50 mg/kg i.p. for three days to rats increased the concentration of noradrenaline in the brain, heart and mesenteric artery (Table 1). A slightly higher dosage of 75 mg/kg also

TABLE 1. *Effect of pyridinolcarbamate (pyrid.) on heart, mesenteric artery and brain biogenic amines*

Tissue	Drug	Dose† mg/kg	Noradrenaline μg/g
Heart	Control	—	1·00±0·08
	Pyrid.	50	1·30±0·11*
Mesenteric artery	Control	—	2·99±0·24
	Pyrid.	50	4·32±0·46*
Brain‡	Control	—	0·34±0·01
	Pyrid.	50	0·49±0·01*
			5-hydroxytryptamine μg/g
Brainstem§	Control	—	0·73±0·07
	Pyrid.	75	0·90±0·05*

† Pyridinolcarbamate was given i.p. as a suspension in a dose of 50 mg/kg or 75 mg/kg once a day for 3 days and rats killed 4 h after the last dose. ‡ Brain refers to the whole brain whereas brainstem includes only the diencephalon, mesencephalon and pons-medulla. § Three groups of 3 mesenteric arteries were pooled for the experiment and 6 hearts or brains assayed individually. Noradrenaline concentrations are ± S.E.M. * Results were statistically different from controls $P<0·05$.

increased the brainstem 5-hydroxytryptamine content. Lower doses or less frequent administration of the drug did not reproducibly alter tissue noradrenaline or 5-hydroxytryptamine content.

Studies on the mechanism of alteration of tissue biogenic amines following pyridinolcarbamate administration were undertaken. Synthesis of noradrenaline was inhibited with α-methyl-*p*-tyrosine 250 mg/kg i.p. Pyridinolcarbamate was then administered twice i.p. in doses of 75 mg/kg two hours apart starting one hour after α-methyl-*p*-tyrosine. Rats were killed three hours after the first dose of pyridinolcarbamate. The results of this experiment were that the normal decline of heart (−30%) and brain (−50%) noradrenaline content produced during this period by the inhibition of catecholamine synthesis was blocked by pyridinolcarbamate. This indicates that the drug may modify metabolism or release of noradrenaline.

Recent studies have indicated that many drugs which modify brain 5-hydroxytryptamine concentrations do so by changing the concentration of its precursor tryptophan (Tagliamonte, Tagliamonte, Perez-Cruet, Stern & Gessa, 1971). We therefore measured the concentration of tryptophan in the brainstem of rats following doses of pyridinolcarbamate which significantly raised the brain 5-hydroxytryptamine content and found no change in tryptophan concentrations.

We found pyridinolcarbamate to be a weak monoamine oxidase inhibitor *in vitro*. At a concentration of 6×10^{-4}M it caused a 35% inhibition and $1{\cdot}3\times10^{-3}$M caused a 57% inhibition of monoamine oxidase. However, at doses which elevate brain 5-hydroxytryptamine content *in vivo* we observed no significant reduction in brain 5-hydroxyindole acetic acid, the deaminated 5-hydroxytryptamine metabolite.

Discussion.—The studies indicate that pyridinolcarbamate raises the concentrations of noradrenaline in the heart mesenteric artery and brain as well as 5-hydroxytryptamine in the brainstem. The mechanism of these increases does not appear to be an augmentation of noradrenaline biosyntheses or an increase in the concentration of 5-hydroxytryptamine precursor. The increased tissue concentrations may, on the other hand, be related to alterations in amine release or metabolism.

Pyridinolcarbamate is a weak *in vitro* monoamine oxidase inhibitor. It is relevant that monoamine oxidase inhibitors have in fact been used to treat hypertension and nialamide, a monoamine oxidase inhibitor, has been reported to be an anti-atherosclerotic agent (Shimamoto, Takeuchi & Ishiaka, 1962; Shimamoto & Sunaga, 1962). However, the hypothesis that the increased amine concentrations were mediated by an effect of pyridinolcarbamate on monoamine oxidase is not supported by the observation that the deaminated metabolite of 5-hydroxytryptamine, 5-hydroxyindole acetic acid, did not fall after pyridinolcarbamate administration as would be expected if monoamine oxidase were significantly inhibited *in vivo*.

Because noradrenaline content was not reduced in the presence of an inhibitor of catecholamine synthesis plus pyridinolcarbamate and tryptophan concentrations in the brain were unaltered by the drug, we suggest the possibility that pyridinolcarbamate impairs release or interferes with utilization of these amines. Although the mechanism of interference with noradrenaline or 5-hydroxytryptamine disposition is not clearly established, it does appear to be somewhat specific since cardiovascular histamine content was not influenced by pyridinolcarbamate (unpublished observation).

The mechanism of action of pyridinolcarbamate is itself somewhat controversial. For example, several studies in animals (Mottonen, Pantie & Nieminen, 1972, Malinow, Mclaughlin & Perley, 1972 and Kritchevsky, Kolimaga, Kim & Tepper, 1972) have shown that cholesterol-induced atheromata or vascular enzyme changes associated with atherosclerosis were unaffected by pyridinolcarbamate. Yet other workers have found dramatic improvement in vascular lesions and in tissue healing and perfusion following pyridinolcarbamate administration (Shimamoto, Malzawa, Yamazaki, Atsumi, Fijita, Ishioka & Sunaga, 1966; Kipshidze, 1972; Lund, 1972). Our studies suggest that the cardiovascular system can be affected directly by an action of pyridinolcarbamate on heart and vascular noradrenaline. Moreover, the fact that this drug modifies central nervous system noradrenaline and 5-hydroxytryptamine concentrations indicates the possibility of a central action. Pyridinolcarbamate does, in fact, have be-

haviourial effects in man (personal communication, G. Craig, Dudley Road Hospital, London). Although this anti-atherosclerotic drug apparently has effects on both the central and peripheral nervous system as well as on atheromatous vascular lesions, no causal relation between these has been established. However, the possibility that there is some direct relation between biogenic amines and atherosclerotic disease is supported by the observations that atherosclerosis induced by cholesterol feeding may alter vascular and cardiac catecholamine metabolism and disposition (Berkowitz, 1972; Gillis & Melville, 1972).

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(*Received December* 19, 1972)