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Antiaccelerator, Coronary Dilator and Certain Other Pharmacologic Actions of New Poly-Methoxyphenyl Derivatives. (23957)

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A specific new pharmacologic activity, the cardiac antiaccelerator effect, was described by Krayer and his associates (for review, *cf.* 1). This activity was found among naturally occurring veratramine and solanum alkaloids (1,2). More recently, a similar effect was described by Margolin and his associates(3) in the case of certain synthetic steroids with alkamine substitutions in position 16. It was conceived that relatively simple polyphenyl molecules with a lipophilic appendage may exhibit similar pharmacologic properties. This paper describes antiaccelerator potency data obtained in the course of screening of a series of such compounds as well as additional pharmacologic and toxicity results obtained with 2 particularly promising compounds of this series.

Methods. The blockade of positive chronotropic effect of epinephrine was tested in rabbit heart Langendorff preparations perfused at 25°C with oxygenated (95% O_2, 5% CO_2) Ringer-Locke by means of an apparatus (4) that permitted measurement of coronary flow. Cardiac rate was measured by means of Palmer drop recorder and amplitude of cardiac beat by means of a light isotonic lever. Epinephrine bitartrate was prepared in Ringer-Locke (5-10 μg/cc) with sodium bisulfite, final concentration 0.1%, acting as antioxidant. Epinephrine was perfused at 1 cc/min. through a side arm provided with a flow meter. The compounds tested were solubilized in weak HCl and injected in 0.1 to 0.2 cc into the perfusion cannula near the heart. The reference compound, veratramine, was solubilized as described by Krayer(5); veratramine doses refer to the base. The positive chronotropic effect of epinephrine amounted in 20 tests to an average of 36 beats/minute and decreased by an average of 5 beats, or 14%, at end of 2 to 4 hours. In actual testing, inhibition of acceleration of 30% or more was considered significant. Anti-accelerator doses effective in 50% of hearts (AED_{50}) were determined from a dose-frequency curve according to the method of Wright(6); 3 to 5 doses were spaced at 0.5 logarithmic intervals, 3 to 5 hearts/dose. Additional measurements of the effect of compounds tested on coronary flow were carried out by the method of Luduena, Miller and Wilt(7). The acute LD_{50} values and their standard error were measured in mice by the method of Miller and Tainter (8). Irritancy was determined in rabbits by means of trypan blue test(9,10). Local anesthetic potency was determined in guinea pigs by the intradermal wheal test(11). The cardiac rate was measured in atropinized dogs under chloralose or barbiturate anesthesia by means of a Palmer Recorder and a pressure transducer(12). The blockade by the com-

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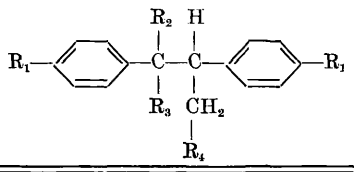
TABLE I. Antiaccelerator and Coronary Dilator Action of Polyphenyl and Reference Compounds.



Compound No.	R_1	R_2	R_3	R_4	Antiaccelerator effect, AED_{50}, μg/heart	Coronary dilatation: Coronary flow increase, %*	Coronary dilatation: % papaverine†
1	OCH_3	—‖	4-methoxyphenyl	NMe_2	31	125	230
2	"	OH	Isobutyl	"	48	105	115
3	"	"	Cyclo-hexyl	"	54	96	
4	"	"	Hexyl	"	50	70	
5	"	"	Isobutyl	1-piperidyl	23	62	≤50
6	"	"	Octyl	NMe_2	≥100		
7‡	H	"	Isobutyl	"	16.5	55	
8‡	H	"	Phenyl	"	≥50	slight	
Veratramine					18	none	.2
Papaverine					≥200	89§	100

* Coronary flow was measured following epinephrine perfusion and inj. of compound tested. Avg increase was calculated at the AED_{50} value.

† Technic of Luduena *et al.*(7). ‡ Known compound. § At 50 μg/heart. ‖ Double bond at the R_2, R_1, carbon.

pounds tested of the epinephrine pressor and of acetylcholine vasodepressor responses, and of the response of nictitating membrane to faradic sympathetic stimulation was measured by conventional methods in dogs and in cats.

Results. The bio-comparison of antiaccelerator potency of the polyphenyl compounds and of veratramine was carried out on the epinephrine-driven isolated rabbit heart. Epinephrine infusion produced well-known inotropic, chronotropic and coronary dilator actions. The active polyphenyl derivatives reduced the positive chronotropic effects of epinephrine without affecting much its inotropic action, in which they resembled veratramine(1). The AED_{50} values for cardiac slowing of all compounds tested, including papaverine and veratramine, are shown in Table I. Several new compounds approximated, in their activity, veratramine. The polyphenyl compounds differed unexpectedly from the veratramine in that several of them caused additional coronary dilatation (Table I), while veratramine produced none as already shown by Krayer(5). The dilator effect of compounds 1, 2 and 5 and of veratramine was tested also by a technic not involving epinephrine(7). There was a good qualitative agreement between the 2 sets of data (Table I). The coronary dilator effect of the new compounds seemed independent of the anti-accelerator action, since it was not parallel with the extent of the latter (Table I) and since, frequently, it obtained at doses which did not slow the heart. In particular, the effect of compound 1 was more than twice that of papaverine (Table I).

Autonomic action, particularly general sympatholytic activity could account for the blockade by the polyphenyl compounds of the chronotropic cardiac effect of epinephrine. Compounds 1 and 5 were found, however, to be incapable of blocking either the pressor effects of epinephrine in dogs or the withdrawal of the nictitating membrane on faradic stimulation of the cervical sympathetic in the cat (Table II); in this they resemble veratramine (5,13) and steroids studied by Margolin *et al.* (3). They were also found incapable of stimulating frog rectus and of cholinergic blockade in the dog (Table II). Another pharmacologic activity that could be involved in cardiac slowing is irritancy and/or local anesthetic action(11,14); both could be expected

TABLE II. Pharmacologic Properties of Compounds 3-Dimethyl-amine 1,1,2-tris-(4-methoxyphenyl)-1-propene and 2,3-bis (4-methoxyphenyl)-5-methyl-1-(1-piperidyl)-3-hexanol, and of Veratramine.

Test	Route	Compound 1	Compound 5	Veratramine
Toxicity in mice, LD_{50}, mg/kg	Intrav.	30 ± 1	31 ± 2.1	3.68 ± .24
	Oral	385 ± 85		13 ± 2.1
Approx. toxicity in unanesthetized dogs, mg/kg	Intrav.	>10	> 5	.3
	Oral	>25	>20	2.0
Irritancy, threshold conc., %		.25	.032	.10
Local anesthetic potency, % procaine		130	25	Convulsions at .2 mg/kg
Sympatholytic activity				
Reversal of epinephrine pressor effect in dogs, mg/kg	Intrav.	Slight blockade	None at 8	
Blockade of nictitating membrane response in cats, mg/kg	"	Slight at 4-10	*Idem*	
Anticholinergic action				
Blockade of acetylcholine depressor effect in dogs, mg/kg	"	None at 10	None at 10	

from polyphenyl compounds(11,14). Actual tests showed that compounds 1 and 5 exhibit local anesthetic potency (Table II). However, compound 1, almost as potent an antiaccelerator as compound 5, is many times weaker than the latter in its irritancy and local anesthetic action. In addition, procaine and a 2-alkoxy thiolbenzoate, a new compound with the anesthetic potency about 100 times that of procaine(15) were tested in the isolated heart for their possible antiaccelerator effects. Neither produced cardiac slowing short of complete AV block and ventricular fibrillation which occurred with about 3 mg per heart of procaine and 25 μg per heart of thiolbenzoate.

It was of interest to test whether or not the antiaccelerator data obtained in the isolated heart would carry over into the intact animal. Compounds 1 and 5 exhibited antiaccelerator action in 15 atropinized and/or vagotomized dogs under pentobarbital or chloralose anesthesia in doses of from 0.3 to 0.5 mg/kg. Epinephrine infusion seemed not essential for this action which agrees with several experiments carried out without epinephrine on the isolated heart. There was little or no effect on mean arterial blood pressure whether in presence or in absence of epinephrine. Compounds 1 and 5, given i.v. in doses of from 0.5 to 2 mg/kg, produced also cardiac slowing in trained unanesthetized dogs (7 experiments). In some instances, the rate decreased from 75 to 50 beats per minute and was maintained at the low level for 1 to 2 hours.

Sympatholytics produce usually compensatory tachycardia. A phenoethiazine sympatholytic(16) was used in trained unanesthetized dogs to produce a tachycardia of up to 200 beats per minute lasting for 5 hours or more. Compounds 1 and 5, 2 mg/kg i.v., or 5-10 mg/kg orally, blocked tachycardia and a rate of 60-70 per minute persisted for up to 4 hours. Electrocardiograms (Lead 2) of the trained unanesthetized animals indicated that the slowing of the rate, whether following experimental tachycardia or in untreated dogs, was a sinus bradycardia with little change in the QRS interval, *i.e.*, with little change in conduction.

Of particular interest in this series of experiments was that while compounds 1 and 5 showed no side actions at doses specified, veratramine produced in intact dogs considerable toxicity (Table II). In anesthetized atropinized dogs (17 experiments) veratramine slowed the heart at levels of 0.02-0.05 mg/kg and produced tremors and analeptic actions. In unanesthetized animals tachycardia, nausea, salivation and excitement appeared with doses of 30 to 40 μg/kg, i.v., and tremors, convulsions and vomiting—with doses of 120-240 μg/kg, i.v., or 2 mg/kg, orally. Two dogs were killed with 0.3 mg/kg, i.v. This indi-

cation that toxicity of veratramine is much higher than that of the polyphenyl compounds was borne out by the LD_{50} data in mice (Table II).

Discussion. The prototype antiaccelerator agent, veratramine, exhibits 2 main pharmacologic actions: it blocks cardiac chronotropic effect of epinephrine, and initiates a complex reflex activity leading to hypotension and bradycardia ("Bezold effect," for review see Krayer and Acheson, 17, and Dawes and Comroe, 18). The present data suggest that the new polyphenyl compounds are antiaccelerators probably devoid of the hypotensive activity. Particularly, compounds 1 and 5 exhibit a specific antiaccelerator action independent of extracardiac sympatholytic and adrenolytic effect, not due to vagal slowing and not related to an effect on conduction which obtains with local anesthetics (*cf.* lack of antiaccelerator action of antiarrhythmic agents and local anesthetics, Krayer *et al.*, 19). Accordingly, the receptor of the "pacemaker tissue"(5) can be suggested as the specific site of antiaccelerator action of veratramine and certain related steroids, as well as of the present polyphenyl compounds. This receptor, although activated by epinephrine and norepinephrine differs in an interesting fashion from other sympathetic receptors in that it cannot be blocked by sympatholytic agents even when used in doses many times those sufficient to abolish vasoconstriction actions of epinephrine and of other sympathomimetics (20,21). Thus, sympatholytics cannot prevent tachycardia provoked by their own vasoconstrictor blockade and hypotension. On the other hand, the polyphenyl antiaccelerators are capable as shown at present, to differentially block the "primitive"(22) receptor of the pacemaker. To further characterize this receptor it should be said that all antiaccelerators described so far possess a relatively rigid polyring nucleus with a secondary or a tertiary N-containing ring, or with a nitrogen-bearing sidechain. Thus, they seem chemically related to endocrine agents; polyphenyl compounds described at present are diethylstilbestrol derivatives, while veratramine, solanum alkaloids and compounds described by Margolin *et al.*(3) are steroids related to testosterone.

Toxicity of veratramine and of steroids described by Margolin *et al.* limited their therapeutic application(3,23). Since the compounds studied at present are many times less toxic than veratramine (Table II) and since they have the additional advantage of being coronary dilators, their clinical trial has been initiated.

Summary. 1. A significant cardiac antiaccelerator activity was found among members of a new synthetic series of polymethoxyphenyl amines. 2. When tested on the isolated, epinephrine driven rabbit heart their potency was found in several instances to be comparable to that of veratramine. 3. They could produce also cardiac slowing in anesthetized and unanesthetized dogs as well as block, in unanesthetized dogs, compensatory tachycardia due to administration of a sympatholytic. 4. Some of the compounds were found to be coronary dilators, in one case 2-3 times more potent than papaverine. 5. Since the polyphenyl compounds were found not to be sympatholytic or adrenolytic, since their action is extra-vagal and independent of their local anesthetic activity, the possibility is suggested they have a specific site of action at the "primitive" pacemaker receptor.

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Influence of Adrenal Hormones on Lactic Acid Content of Rat Brain Tissue. (23958)

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An increase in lactic acid content of brain tissue in hypoxic animals has been established by various investigators(1-4). When brain tissue is analyzed one to 3 hours post mortem the lactic acid level may increase 4- to 5-fold (4). This probably is the result of endogenous enzymatic activity on lactic acid precursors such as glycogen, glucose or other carbohydrate material. There is considerable evidence to show that adrenal steroids are involved, in a permissive manner, in carbohydrate metabolism(5). This fact coupled with the almost exclusive use of carbohydrate in normal brain metabolism, suggested a study of the lactic acid content in brain tissue as influenced by hypoxia and adrenal hormones.

Methods. Male Sprague-Dawley rats weighing about 350 g were used. They were divided into 2 main groups; intact and adrenalectomized. All animals received Purina Laboratory Chow and water *ad libitum*, with the adrenalectomized animals receiving physiological saline in place of water. Four days post-operatively, the animals were placed into 2 subgroups; a ground level group which will be referred to as the "non-hypoxic" group and an altitude group which will be designated as the "hypoxic" group. Hypoxia was produced by taking the animals to a simulated altitude of 30,000 feet (225.6 mm Hg) for a period of 105 minutes in a decompression chamber. This time and altitude were found to give a large increase in lactic acid content of brain tissue in intact control animals. At the end of experimental or control period, the animals were decapitated in their respective environments; *i.e.*, at ground level or at altitude. The decompression chamber was routinely returned to ground level within 5-6 minutes following decapitation. All heads were left intact at room temperature (23°-27°C) for a period of 3 hours, after which the brains were removed, blotted of excess blood, and homogenized in glass homogenizers with 10% trichloracetic acid (10% homogenate). The lactic acid content of the whole brain was determined by the method as described by Van Fossan(4). Lactic acid content of brains from intact and adrenalectomized animals was determined under non-hypoxic and hypoxic conditions to obtain control data. In comparison, brain lactic acid content of adrenalectomized animals receiving the following adrenal hormones and dosages was determined: (a) epinephrine, 0.15 mg in sesame oil given subcutaneously 15 minutes prior to ascent to altitude; (b) corticosterone, 1.0 mg in saline given subcutaneously at 4 two-hour