Table II—Recoveries of Drotaverine from Spiked Rat and Dog Plasma [a]

Drotaverine Concentration, μg/mL	Recovery from Rat Plasma, %	Recovery from Dog Plasma, %
0.25	83.2 ± 4.7	82.8 ± 6.8
0.50	77.0 ± 9.1	84.4 ± 5.2
1.00	82.3 ± 6.5	85.8 ± 8.8
1.50	89.8 ± 6.0	87.9 ± 4.9
2.00	94.3 ± 7.3	88.7 ± 7.1

[a] Mean ± *SD*; $n = 8$.

plasma extracts from a rat given 60 mg/kg of drotaverine, and from a dog given 30 mg/kg of drotaverine, are shown in Figs. 2 and 3.

The drotaverine ultraviolet spectrum has maxima at 242, 302, and 350 nm wavelengths. A variable-wavelength UV detector set at 302 nm was used to eliminate background contributions to the drotaverine peak at 242 nm in blank plasma extracts. The intensity of the maximum at 350 nm is lower than that of the maximum at 302 nm. The detection limit was found to be 50 ng of drotaverine/mL of plasma.

Data in Table I show the linearity of the assay method. A linear regression analysis was performed for the calibration curve prepared from drotaverine-spiked plasma samples. It resulted in the equation $y = 0.2912x + 0.0038$, where y is the ratio of the peak height of the drug divided by the peak height of the internal standard, and x is the drug concentration in the plasma. As evidenced by the experimental data, peak heights were proportional to the drotaverine concentrations for the range examined (from 0.25 to 2 μg of drotaverine/mL of plasma).

Recoveries of drotaverine were nearly quantitative at plasma concentrations of 0.25–2.0 μg/mL, with average recoveries ranging from 77.0 to 94.3% and coefficients of variation ranging from 4.7 to 9.1% (Table II).

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Influence of α- and β-Adrenergic Antagonists on Dopamine-Induced Responses in the Isolated Heart of *Mercenaria mercenaria*

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Abstract □ Agents with predominantly adrenergic antagonistic properties (the α-blockers, phentolamine and tolazoline, and the β-blocker propranolol), and those with suspected α-blocking capabilities [hydralazine and 3,3′-(4,4′-biphenylene)bis(2,5-diphenyl-2*H*-tetrazolium chloride) (neotetrazolium chloride); I] were added individually to a bath containing an isolated *Mercenaria mercenaria* heart. Two and one-half minutes later, dopamine was added to the bath as the second drug and cardiac responses were noted. Pretreatment with saline controls, followed by dopamine 2.5 min later, produced results that were identical with those which occurred after the administration of dopamine alone, *i.e.*, marked stimulation and cardiac arrest. Pretreatment with phentolamine and I were the only procedures that prevented dopamine-induced cardiac arrest. Phentolamine, tolazoline, and hydralazine generally produced positive inotropic responses when initially added to the bath, whereas propranolol mimicked the effects caused by the addition of dopamine alone or after saline pretreatment. The *M. mercenaria* heart appears to possess an adrenergic receptor of an α-configuration.

Keyphrases □ α- and β-Adrenergic antagonists—dopamine-induced response, heart of *Mercenaria mercenaria* □ Dopamine—α- and β-adrenergic antagonists, effect on heart of *M. mercenaria*, pretreatment controls.

A number of studies have suggested the presence of an adrenergic receptor in the smooth muscle of the heart of *Mercenaria mercenaria*, the hard-shelled clam or quahog. Welsh and Taub (1) administered various drugs to the isolated heart of *M. mercenaria* and noted that epinephrine evoked negative inotropic responses and tachyphylaxis. Fujita and Mann (2) reported similar responses with norepinephrine under comparable experimental conditions. Ciuchta and Mann (3) examined the relative effectiveness of ephedrine isomers in preventing the onset of norepinephrine-induced tachyphylaxis in the *M. mercenaria* heart and found that the *l*-isomer was the most potent, the racemate less potent, and the *d*-isomer least potent. Orzechowski and Mann (4) performed a similar experiment with amphetamine isomers and observed that norepinephrine-induced tachyphylaxis was blocked most effectively by the *d*-isomer, while the *d,l*- and *l*-isomers were equal but less potent. When higher doses of amphetamine isomers were given, norepinephrine elicited positive inotropic responses, suggesting that these agents act upon common receptor sites. It was further speculated that amphetamine, like ephedrine, may block cardiac inhibitory sites on which norepinephrine acts, thus allowing ephedrine to cause stimulation.

Dopamine, which is present in *M. mercenaria* nervous tissue (5), appears to activate excitatory receptors in the clam heart in contrast to epinephrine and norepinephrine, which affect inhibitory receptors. Tachyphylaxis also occurs in the *M. mercenaria* heart after successive, equal doses of dopamine[1].

The purpose of this study was twofold:

1. To determine whether the α-blockers (tolazoline, phentolamine, and hydralazine) and the β-blocker (propranolol) could prevent the excitatory effects of dopamine on the isolated *M. mercenaria* heart, thereby elucidating the nature of its adrenergic receptor;

2. To ascertain whether the hypotensive agent, 3,3′(4,4′-biphenylene)bis(2,5-diphenyl-2*H*-tetrazolium chloride) (neotetrazolium chloride; I), which has both adrenergic α-blocking and gangliolytic actions (6–8), could antagonize

[1] Unpublished results.

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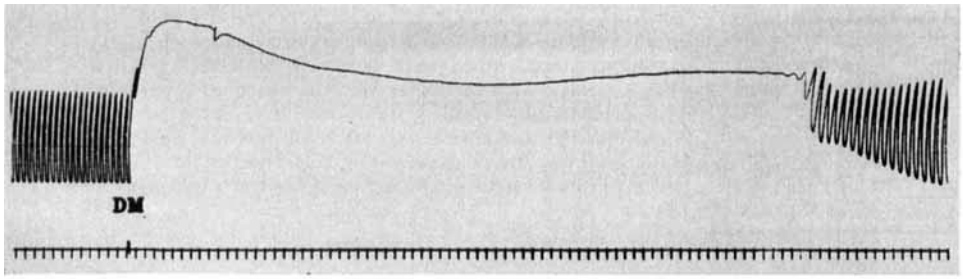

Figure 1—*Effect of dopamine hydrochloride (4 mg) on isolated* M. mercenaria *heart; time interval is 5 s.*

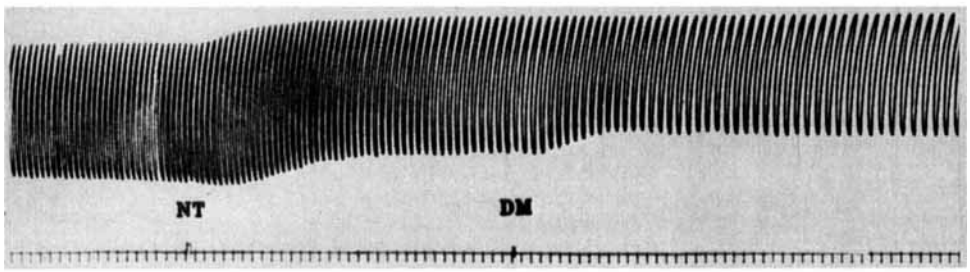

Figure 2—*Preadministration of 3,3′-(4,4′-biphenylene)bis[2,5-diphenyl-2H-tetrazolium chloride] (8 mg) followed by dopamine hydrochloride (4 mg);* $p = <0.05$.

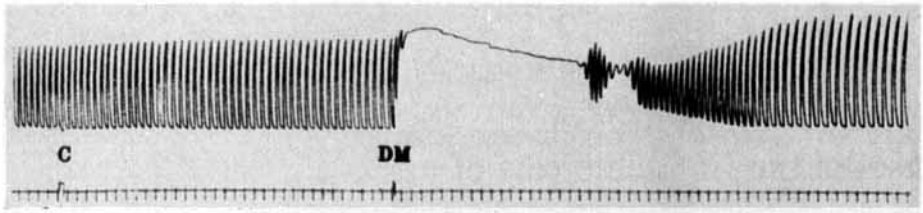

Figure 3—*Preadministration of a vehicle control for 3,3′(4,4′-biphenylene)bis[2,5-diphenyl-2H-tetrazolium chloride] (0.8 mL) followed by dopamine hydrochloride (4 mg);* $p < 0.001$.

Table I—Average Percent Inhibition[a] Within 1 min After Challenging the Isolated *M. mercenaria* Heart with a Test Drug and within 1 min After the Addition of Dopamine Hydrochloride

Compound	*n*	Dose	Average Inhibition after 1 min, % No Challenge	Average Inhibition after 1 min, % Dopamine Hydrochloride Challenge	*p*
Phentolamine mesylate	7	15 mg	36	59	<0.001[b]
Tolazoline hydrochloride	8	15 mg	24	100	—
Propranolol hydrochloride	8	15 mg	100	—[d]	—
Hydralazine hydrochloride	8	15 mg	17	100	—
Saline control	8	2.5	26	100	—
3,3′-(4,4′-Biphenylene)bis(2,5-diphenyl-2*H*-tetrazolium chloride)	15	8	22	32	<0.05[b]
Solvent control	6	0.8 ml	0	100	<0.001[c]

[a] The greatest degree of inhibition within 1 min after drug challenge. [b] Statistically significant compared with saline control. [c] Statistically significant compared with neotetrazolium chloride-treated group. [d] Contractility failed to return after propranolol administration; dopamine administration caused greater spasticity.

the effects of dopamine on the *M. mercenaria* heart. Positive results obtained with I and the other adrenergic α-blockers would presume an adrenergic α-receptor as the site upon which dopamine acts, because the *M. mercenaria* heart is devoid of autonomic ganglia.

EXPERIMENTAL SECTION

Animals—*M. mercenaria*[2] were maintained in a 378.4-L aquarium filled with artificial sea water[3] for at least 2 d prior to use.

Apparatus—The heart was isolated according to the methods of Welsh and Taub (1) and Wait (9). After cracking the shell with a hammer, the heart was removed and attached to a hook embedded in a plastic rod. The rod was then lowered into an aerated[4] glass bathing chamber containing 40 mL of sea water. A thread extending from the apex of the ventricle to a detecting head[5] activated a transducer[6] and physiograph[7] to record contractility.

Materials—When contractions were normal, a glass syringe[8] delivered either 0.8 mL of neotetrazolium chloride[9], 0.8 mL of the vehicle control[9], 2.5 mL of the test drug, or 2.5 mL of a saline control[10], followed 2.5 min later by the addition, using a second syringe[8], of 4 mg (0.1 mL) of dopamine hydrochloride[11]. The test drugs were prepared in the following concentrations: phentolamine mesylate[12], tolazoline hydrochloride[13], and propranolol hydrochloride[14] at 0.6%; hydralazine hydrochloride[15] and I[9] at 1.0%.

Pretreatment Dose Regimen—A 15-mg dose of each agent was administered 2.5 min prior to dopamine challenge (except for I, the dose of which was 8 mg). A 2.5-mL aliquot of the saline control was added to the bath; this volume was equivalent to that of each test drug, except for I. Because the latter compound utilized a vehicle control (5% ethyl alcohol and 20% propylene glycol in distilled water) and a lower dosage than the rest, it has a control volume of only 0.8 mL.

[2] Obtained locally; shell lengths 10–14 cm.

[3] Instant Ocean; Aquarium Supplies, Mentor, Ohio. Maintained at 24–26°C; specific gravity, 1.025.

[4] Oscar's 55 air pump; Berkeley, Calif.

[5] Isotonic myograph, Model 1127; Narco Bio-systems, Inc., Houston, Tex.

[6] Isotonic myograph transducer, MK II; Narco Bio-systems, Inc.

[7] Physiograph Four-B; Narco Bio-systems, Inc.

[8] B-D mL tuberculin syringe with a 1.27-cm, 26-gauge needle; B-D 2-ml Multifit syringe with a 1.27-cm, 26-gauge needle.

[9] I, Cat. No. 102436, Lot No. 1285, ICN Pharmaceuticals, Inc., Life Sciences Group, Cleveland, Ohio. (In distilled water as 1% I, 5% ethyl alcohol (95%), and 20% propylene glycol.) Added to the bath in a volume of 0.8 mL and controlled with equivalent volumes of a distilled water vehicle containing 5% ethyl alcohol and 20% propylene glycol.

[10] Normal saline, 0.9%, Lot No. 94-851-DE-5; Abbott Laboratories, North Chicago, Ill.

[11] Intropin (dopamine hydrochloride), 5 mL ampules containing 40 mg/mL, Lot Nos. A0022, A9158; Arnar-Stone Laboratoris, Inc., McGaw Park, Ill.

[12] Regitine (phentolamine mesylate), Lot. No. B66-R-113; obtained through the courtesy of Ciba Pharmaceutical Co., Div. of Ciba-Geigy Corp., Summit, N.J.

[13] Priscoline (tolazoline hydrochloride), Lot No. A-7856; obtained through the courtesy of Ciba Pharmaceutical Co., Div. of Ciba-Geigy Corp.

[14] Inderal (propranolol hydrochloride), Lot No. R47736A; obtained through the courtesy of Ayerst Laboratories, Inc., New York, N.Y.

[15] Apresoline (hydralazine hydrochloride), Lot No. N-2059; obtained through the courtesy of Ciba Pharmaceutical Co., Div. of Ciba-Geigy Corp.

RESULTS AND DISCUSSION

The typical response elicited by dopamine (4 mg) on the isolated *M. mercenaria* heart bathed in 40 mL of artificial sea water is shown in Fig. 1. The heart stopped contracting at once when dopamine was added to the bath, but recovered its contractility within 6 min. When I (8 mg) was added to the bath, 2.5 min before dopamine challenge, it not only prevented cardiac arrest due to dopamine, but also produced a negative inotropic response that was mimicked by the subsequent addition of dopamine (Fig. 2). By contrast, preadministration of the vehicle control for I, in a volume comparable to that of the test drug (0.8 mL), failed to prevent cardiac arrest caused by dopamine; however, the recovery time for the return of contractility was shortened over that induced by dopamine administration alone (Fig. 3).

The average percent of cardiac inhibition attributed to the test drugs and to dopamine challenge is presented in Table I. The number of animals used in each group ranged from 6 to 15. The percent inhibition was calculated by measuring the amplitude of the peak at the moment of test drug challenge and dopamine challenge, and the lowest amplitude within 1 min after administering each agent; *i.e.*, the response recorded in Fig. 1 after the addition to the bath of dopamine alone represents 100% inhibition.

Although each agent, except the solvent control, caused some degree of cardiac inhibition within 1 min of its administration (Table I), propranolol produced the most dramatic and consistent inhibitory responses. Immediately after the addition of the β-blocker to the bath, the heart lost contractility and became spastic, an excitatory response that was identical with that observed after the administration of dopamine alone (Fig. 1). Two and one-half minutes later, the addition of dopamine produced an even greater response.

Initial administrations of phentolamine, tolazoline, and hydralazine yielded consistently positive inotropic responses during the 2.5-min period prior to the addition of dopamine, whereas I caused variable effects (*i.e.*, positive, negative, or no alteration of the inotropic response) prior to the addition of dopamine. Phentolamine mesylate, an α-adrenergic blocker, and I, an experimental hypotensive agent with presumed α-adrenergic blocking activity, appear to act on an adrenergic receptor of an α-configuration within the *M. mercenaria* heart to block the stimulatory effects of dopamine. Of the two compounds, I is the more potent in this respect.

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Identification and Determination of the *S*-Methyl Metabolite of Captopril in Human Plasma by Selected-Ion Monitoring Gas Chromatography–Mass Spectrometry

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Abstract □ The *S*-methyl metabolite of captopril was identified and determined in human plasma by positive chemical ionization selected-ion monitoring gas chromatography–mass spectrometry. After oral administration of 100 mg of captopril to healthy subjects, the maximum plasma level was 60–114 ng/mL. These data for the *S*-methyl metabolite of captopril were correlated to total and unchanged captopril levels.

Keyphrases □ Captopril—identification and determination of the *S*-methyl metabolite in human plasma, gas chromatography-selected-ion monitoring mass spectrometry □ Gas chromatography-selected-ion monitoring mass sepectrometry—determination of the *S*-methyl metabolite of captopril in human plasma after oral administration

Captopril (1-[(2*S*)-3-mercapto-2-methylpropronyl]-L-proline; I) (1, 2) has received considerable attention as an orally active angiotensin-converting inhibitor (3) that is effective in lowering arterial blood pressure (4). The absolute bioavailability and pharmacokinetics of [^{14}C]captopril given orally and intravenously have been reported previously (5). Analysis of the kinetic data suggested that captopril is extensively partitioned into tissue. The interconversion of protein-bound captopril with non-protein-bound mixed disulfides has been suggested as a biotransformation pathway that may extend the pharmacological effects of captopril (5, 6).

Gas chromatography–mass spectrometry (GC–MS) selected-ion monitoring (SIM) methods have been reported for the determination of unchanged captopril in whole blood (7) and total captopril in plasma (8). Total captopril includes unchanged drug, the disulfide dimer of the parent drug, and mixed disulfides with endogenous thiol-containing compounds, *e.g.*, glutathione and cysteine. Recently, the *S*-methyl metabolite of captopril (II) has been identified as a urinary metabolite in humans (9) and rats (6). A GC–MS SIM method was developed to measure the plasma level of the *S*-methyl metabolite (II) after oral administration of captopril. The plasma levels of the metabolite II from samples collected from healthy human subjects after the administration of a single 100-mg tablet of captopril were compared with the free captopril blood levels and total captopril plasma levels determined as IV by GC–MS SIM methods previously reported (7, 8).

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