Biochemical Pharmacology, Vol. 25, pp. 1645–1651. Pergamon Press, 1976. Printed in Great Britain.

EFFECT OF SYMPATHOMIMETIC AMINES AND MONOAMINE OXIDASE INHIBITORS ON PROTEIN SYNTHESIS IN RAT HEART*

SAMUEL MALLOV

Department of Pharmacology, State University of New York, Upstate Medical Center, Syracuse, N.Y. 13210, U.S.A.

(*Received* 8 *August* 1975; *accepted* 5 *November* 1975)

Abstract—The sympathomimetic amines, epinephrine, norepinephrine and isoproterenol, were administered in large doses to rats and the effects on the rates of protein synthesis in isolated perfused hearts of these animals were determined. Protein synthesis was found to be significantly elevated after each drug. Since it had been reported that the hydrazine monoamine oxidase inhibitors reduced or prevented the cardiac lesions caused by the administration of large doses of isoproterenol to experimental animals, the effects of two such agents on the epinephrine-induced increase in cardiac protein synthesis were investigated. Iproniazid had no effect and phenelzine reduced the elevation in protein synthesis to a small degree only. The possible mechanisms by which high concentrations of sympathomimetic amines promote cardiac protein synthesis are discussed.

The administration of large doses of sympathomimetic amines has been found to produce cardiac hypertrophy [1–4] and/or cardiac necrosis [5–10] in a number of different species of experimental animals. The clinical counterparts of these laboratory phenomena occur in some patients with high endogenous levels of epinephrine and norepinephrine due to the presence of pheochromocytomas [11–15] and in a number of patients treated for shock by the intravenous infusion of norepinephrine [11–16].

In an initial study of the mechanism of catecholamine-induced cardiac hypertrophy, we found that incubated thin sections of left ventricle from hearts of rats that had been injected subcutaneously one or more times with large doses of suspensions of epinephrine, norepinephrine or isoproterenol in oil manifested significantly higher rates of protein synthesis than did similar sections from hearts of appropriate controls [17]. The direct addition of catecholamines to the incubation medium did not, however, alter the rate of protein synthesis in the sections. We therefore suggested that the increased cardiac protein synthesis in the animals treated with catecholamines was not due to a direct effect of these agents on protein synthetic processes but to (a) an indirect effect on the latter provoked by increased cardiac rate, contractile force or work, or to (b) an adaptive or reparative response of the heart to cardiac injury produced by the drugs. The tissue slice was used as a simple model for the evaluation of the effect of drugs on protein synthesis in the heart that permitted the maintenance of arbitrary constant levels of protein precursor amino acids in the environment as well as the avoidance of complicating effects of nervous and hormonal stimuli at the time the rates of protein synthesis were measured. However, since results obtained with tissue slices may not necessarily reflect the situation in intact animals, an additional model has been chosen for the study—the isolated perfused heart. We report here the qualitatively similar results obtained with this preparation.

Various monoamine oxidase (MAO) inhibitors, particularly those that are derivatives of hydrazine, have been reported to decrease the severity and frequency of the necroses produced in the hearts of experimental animals by the administration of large doses of isoproterenol [18–20] or by coronary artery ligation [21, 22]. Clinically, these agents have been found to reduce the frequency and severity of attacks of angina pectoris [23–26] and to increase subjective exercise tolerance [27]. We therefore thought that, if our observed catecholamine-induced increases in cardiac protein synthesis were consequences of cardiac injury, MAO inhibitors might act to inhibit such increases. The results of a study of the effects of two representative MAO inhibitor compounds on epinephrine-induced elevation of protein synthesis in rat hearts are presented.

MATERIALS AND METHODS

Animals. Male Sprague–Dawley rats, 350–400 g in weight, purchased from Holtzman Farms, Madison, Wis., and maintained on a diet of Agway rat and mouse diet pellets *ad lib.* except when otherwise noted, were used in all experiments.

Materials. L-Epinephrine bitartrate, L-norepinephrine (Arterenol) bitartrate, L-isoproterenol bitartrate, heparin, and nonradioactive amino acids were purchased from the Sigma Chemical Co., St. Louis, Mo. [^{14}C-U]L-phenylalanine, [4, 5-^{3}H]L-leucine and [^{3}H-G]inulin were obtained from the New England Nuclear Co., Boston, Mass. Iproniazid phosphate was purchased from the Aldrich Chemical Co., Inc., Milwaukee, Wis., and phenelzine sulfate was kindly supplied to us by the Warner–Lambert Research Institute, Morris Plains, N.J.

* This work was supported by USPHS grant HL 13392.

Administration of drugs in vivo. Suspensions of the bitartrate salts of L-epinephrine, L-norepinephrine or L-isoproterenol in oil were prepared by grinding weighed quantities of the salts in measured volumes of cottonseed oil by means of a mortar and pestle at the times of injection. Doses of either 2.75 or 5.50 μmoles/kg of the amines (5.5 μmoles of epinephrine is equal to 1 mg of epinephrine base) were injected s.c. into the experimental rats three times during a 24-hr period (at 9 a.m., 5 p.m. and 9 a.m. the following morning), while equal volumes of oil vehicle alone were injected at the same times into control animals. Food and water were permitted *ad lib.*

In some experiments, rats, injected with epinephrine on the above schedule, were also injected s.c. with a monoamine oxidase inhibitor—either phenelzine sulfate or iproniazid phosphate—in aqueous solution, in doses of 25 mg/kg, three times, once at 5 p.m. on the day prior to the start of the catecholamine injections and also at the same times as the first and third catecholamine injections. Controls were injected with equal volumes of saline. The rats in these experiments were fasted during the entire period of drug administration.

All animals were killed 5 hr after the last injection.

Heart perfusions. The rats were injected i.p. with 5 mg heparin in 0.2 ml saline solution 0.5 to 1 hr before they were killed. At the latter time they were anesthetized by the i.p. injection of sodium pentobarbital, after which their hearts were quickly removed and placed into Petri dishes containing ice-cold perfusion medium, causing immediate cessation of beating. The hearts were then dissected free of extraneous tissues, and after the three large arterial branches of the aortic arch were ligated, attached via the aortic stumps to glass cannulas of the perfusion apparatus. Perfusions were begun immediately at a temperature of 37° and the hearts promptly resumed beating. The first 15–20 ml of perfusion fluid expelled from each heart was discarded as washout fluid, and the heart was then placed into its appropriate chamber in the perfusion apparatus without interruption of perfusion. Recirculating perfusion was then carried out for 1–2 hr via the aorta through the coronary arteries. These Langendorff preparations performed no or negligible external work and were capable of beating vigorously for 4 hr. Heart rates ranged from 200 to over 300 beats/min throughout the perfusion periods. Any heart with an initial rate blow 200 beats/min was discarded. The perfusion apparatus employed was a modification of that of Neeley *et al.* [28].

Each heart was perfused with 100 ml of Krebs–Ringer bicarbonate buffer solution at pH 7.4 through which was continuously bubbled a mixture of 95% O_2 plus 5% CO_2, and which contained glucose (10 mM), 20 non-radioactive amino acids (in concentrations approximately equal to those in normal rat plasma and previously reported [19]), and either [^{14}C]-L-phenylalanine (5 μCi) or [4, 5-^{3}H]L-leucine (50 or 100 μCi) and sometimes [^{3}H]inulin (50 μCi). The radioactive inulin was added in those experiments in which intracellular specific activities of free [^{14}C]phenylalanine were to be determined and tissue extracellular space had to be assessed. In some experiments with hearts from untreated rats, L-epinephrine bitartrate was added directly to the perfusion medium at the start, in 1-hr perfusions, to give a concentration in the medium of 0.11 μg (base)/ml (6×10^{-7} M), and again, in the same quantity, at the beginning of the second hr, in 2-hr perfusion experiments. Greater concentrations tended to produce arrhythmias and therefore were not employed. In these experiments, ascorbic acid was also added to the perfusion fluid (5 mg/100 ml) to inhibit oxidation of the added epinephrine.

The perfusion flow rate through all hearts was maintained at exactly 10.2 ml/min. Initial experiments indicated that the rate of protein synthesis in the perfused hearts increased to a maximum value as the flow rate was raised. The flow rate chosen permitted maximum protein synthesis yet did not cause deterioration of the hearts. At higher flow rates it was observed that a number of hearts became distended with fluid and beat weakly toward the end of the perfusion period, probably as a result of leakage of perfusion fluid through the aortic valves. At the fixed flow rate employed, perfusion pressures varied from 50 to 70 mm Hg in different hearts. It was established, however, that as long as the flow rate was kept constant, these variations in pressure did not significantly affect the rates of protein synthesis. Thus, in an experiment in which pressures were deliberately varied but the flow rate was maintained at 10.2 ml/min, the radioactivities of the cardiac proteins after 2 hr of perfusion with radioactive phenylalanine were 2036, 2249, 2168 and 1996 dis./min/mg of protein at flow rates of 52, 61, 65 and 70 mm Hg respectively.

In each experiment, two to four hearts were perfused simultaneously. A fresh batch of perfusion fluid was made up for each experiment, and equal volumes were distributed to the chambers to contain the perfused hearts; therefore, although the specific radioactivities of the radioactive amino acids in the perfusion fluid varied slightly in different experiments, they were exactly the same for all hearts perfused in any given experiment. The results obtained with hearts from drug-treated rats were then compared with those from the simultaneously perfused hearts of control animals.

Protein radioactivity. After perfusion, the hearts were removed from the apparatus and the left ventricles plus intraventricular septa were quickly dissected out, rinsed in ice-cold saline solution, blotted well with filter paper, and divided into sections for the determination of protein radioactivity, water content and intracellular specific activity of free radioactive amino acid. Each section of heart used for protein isolation was placed into 5 ml of 5% trichloroacetic acid (TCA) (w/v), homogenized by means of a Polytron homogenizer (Brinkmann Instrument Co.), and centrifuged. The sediment was washed with cold TCA, then TCA at 95° containing 1 mg/ml of unlabeled phenylalanine or leucine, dissolved in 0.4 M NaOH and re-precipitated with 50% TCA, washed with 3:1 ethanol–ether, then ether alone, and finally dried under a stream of nitrogen. Two 5- to 6-mg samples of the dried protein from each heart were weighed on an analytical balance, solubilized in 0.2 ml of NCS tissue solubilizer (Amersham/Searle Co.) and counted in the presence of a toluene fluor containing 2,5-diphenyloxazole and 1,4-bis-[2-(5-phenyloxazolyl)] benzene (Spectrafluor, Nuclear Chicago) in a Nuclear Chicago Mark I scintillation counter. Cor-

rections of counts for quenching were made with the use of external standards and radioactivities were calculated as dis./min/mg of dry protein.

Intracellular specific radioactivities. Intracellular specific radioactivities of [^{14}C]phenylalanine were calculated as follows, from determined values of total tissue and extracellular phenylalanine specific radioactivities, extracellular spaces and intracellular spaces, according to the procedure of Scharff and Wool [29]:

$$\text{Intracellular specific activity} = \frac{\text{(specific activity in total tissue water)} - \text{(specific activity in extracellular water)} \times \text{(fraction of cell water that is extracellular)}}{\text{(fraction of cell water that is intracellular)}}$$

It was assumed that labeled and unlabeled phenylalanine and inulin in the perfusion fluid were in equilibrium with the same substances in the tissue extracellular fluid. All determinations were made at the end of the perfusion periods. To determine total tissue water, small sections of left ventricle were blotted, weighed, placed in an oven at 100° for 48 hr, cooled in a desiccator, and weighed again. Sections of left ventricle were subjected to the procedure of Scharff and Wool [29] for extraction of total tissue phenylalanine and inulin. This involved placement of the tissues in boiling water for 5 min, precipitation of the proteins with sulfosalicylic acid, and filtration. Extraction was found to be maximum when the tissue was minced before being immersed in the boiling water, and a water-clear filtrate could be obtained if the tissues in sulfosalicylic acid solution were put in the freezer overnight and thawed on the following day before being filtered through Whatman No. 2 filter paper. Aliquots of the filtrates were removed for counting [^{14}C]phenylalanine and [^{3}H]-inulin (double labeling) in a scintillation counter and for determination of total phenylalanine concentrations with the aid of an amino acid analyzer. Samples of perfusion fluid were also obtained after perfusion of the hearts. One ml of 20% (w/v) sulfosalicylic acid was added to 3 ml fluid to precipitate any protein present. After filtration, aliquots of the clear filtrates were removed for the counting of [^{14}C]phenylalanine and [^{3}H]-inulin, and the determination of phenylalanine concentration. Because of the time involved in determination of amino acids with the amino acid analyzer, intracellular specific activities of the labeled amino acids were not determined in all experiments. When they were, the rate of incorporation of amino acid precursors into heart protein was expressed not only as dis./min/mg of protein but also as μmoles of amino acid incorporated into 1 g of cardiac protein/perfusion period.

Table 2. Effect of sympathomimetic amines *in vitro* or *in vivo* on intracellular specific activities of [^{14}C]phenylalanine in isolated perfused rat hearts

Experiments	Specific activities of [^{14}C]phenylalanine (μCi/μmole)		
	Perfusion fluids	Control hearts	Hearts exposed to amine
6, 8, Table 1	0.490 ± 0.051*	0.448 ± 0.036	0.489 ± 0.042
4, 5, 7, 9, 10, Table 3	0.502 ± 0.043	0.499 ± 0.028	0.486 ± 0.023
1, 3, 7, Table 4	0.475 ± 0.026	0.496 ± 0.034	0.445 ± 0.051
1, 9, Table 5	0.492 ± 0.021	0.453 ± 0.031	0.477 ± 0.025

* Mean ± standard error of mean. Specific activities were determined as described in Methods.

RESULTS

Epinephrine in vitro. The activity of epinephrine added *in vitro* was immediately manifested by an increase in heart rate. In every experiment, it was found that the addition of epinephrine to the perfusion fluid caused a small decrease in the rate of protein synthesis (Table 1). This was true whether radioactive leucine or phenylalanine was used as protein precursor and whether perfusion was carried out for 1 or 2 hr. The decrease in rate of protein labeling in hearts exposed to epinephrine *in vitro* was not due to an effect of the drug on intracellular specific actactivities of the radioactive amino acid, since such activities were not significantly different in epinephrine-treated and control hearts in those experiments in which they were determined (Table 2). A decreased rate of incorporation of amino acid into the protein of hearts exposed to epinephrine was manifested in the experiments in which intracellular specific activities were included in the calculations and the results expressed as μmoles phenylalanine/g of protein as well as dis./min/mg of protein (experiments 6–8, Table 1).

Table 1. Effect of epinephrine *in vitro* on rate of protein synthesis in isolated perfused rat hearts*

Expt.	Labeled amino acid	Perfusion time (hr)	Radioactivity of cardiac protein			Incorporation of labeled amino acid into cardiac protein		
			Control (dis./min/mg)	Epinephrine* (dis./min/mg)	Change (%)	Control (μmoles/g)	Epinephrine* (μmoles/g)	Change (%)
1	[^{3}H]leu	1	11.202	9.926	−11.4			
2	[^{3}H]leu	1	12.009	11.669	−2.8			
3	[^{3}H]leu	1	13.212	12.803	−3.1			
4	[^{3}H]leu	1	14.995	11.214	−25.2			
5	[^{3}H]leu	1	12.431	10.937	−12.0			
6	[^{14}C]phe	1	1.322	990	−25.1	1.49	1.05	−29.5
7	[^{14}C]phe	2	1.965	1.499	−23.7	2.70	0.98	−63.7
8	[^{14}C]phe	2	2.172	1.885	−13.2	2.30	2.18	−5.2
Mean					−14.6			−32.8
± S.E.M.					±3.26			±16.97

* The same quantity of L-epinephrine bitartrate was added to the perfusion fluid at the start of each hr of perfusion. The concentration of epinephrine in the perfusion medium after the first addition was 0.11 μg (base)/ml (6×10^{-7} M). Hearts were perfused in the presence of a radioactive amino acid, unlabeled amino acids and glucose.

Table 3. Effect of administration of epinephrine *in vivo* on rate of protein synthesis in isolated perfused rat hearts

Expt.	Labeled amino acid	Perfusion time (hr)	Radioactivity of cardiac protein: Control (dis./min/mg)	Radioactivity of cardiac protein: Epinephrine* (dis./min/mg)	Radioactivity of cardiac protein: Change (%)	Incorporation of labeled amino acid into cardiac protein: Control (μmoles/g)	Incorporation of labeled amino acid into cardiac protein: Epinephrine* (μmoles/g)	Incorporation of labeled amino acid into cardiac protein: Change (%)
1	[^{3}H]leu	1	13,869	17,776	+28.2			
2	[^{3}H]leu	2	21,149	34,352	+63.3			
3	[^{14}C]phe	1	1,093	1,373	+25.6			
4	[^{14}C]phe	1	1,161	1,540	+32.6	1.18	1.43	+21.3
5	[^{14}C]phe	1	1,002	1,128	+12.6	1.08	1.44	+32.4
6	[^{14}C]phe	1	975	1,250	+28.2			
7	[^{14}C]phe	2	2,324	3,200	+37.7	1.81	2.36	+30.3
8	[^{14}C]phe	2	2,428	4,788	+97.2			
9	[^{14}C]phe	2	2,930	3,907	+33.3	2.10	2.72	+29.6
10	[^{14}C]phe	2	1,954	3,228	+65.2	1.96	5.07	+158.6
11	[^{14}C]phe	2	2,226	2,673	+20.1			
12	[^{14}C]phe	2	1,939	2,915	+50.3			
Mean					+41.2			+54.4
± S.E.M.					±6.90			±26.11

* Epinephrine, in suspension in oil, was injected s.c. in a dose of 5.5 μmoles/kg (1 mg base/kg) three times during a period of 24 hr into the experimental rats while controls were injected with equal volumes of oil vehicle alone. Hearts were removed 5 hr after the third injection and perfused in the presence of a radioactive amino acid, unlabeled amino acids and glucose.

Epinephrine in vivo. In contrast to the depression of cardiac protein synthesis caused by the presence of epinephrine *in vitro*, a consistently higher rate of protein synthesis occurred in perfused hearts from rats that had been injected with epinephrine than in the corresponding hearts from vehicle-treated controls (Table 3). The mean increase was 41 per cent. Again the result was the same whether radioactive phenylalanine or leucine was used as protein precursor and whether the hearts were perfused for 1 or 2 hr. When intracellular specific activities were taken into consideration (Table 2), similar increases in protein synthesis were manifested (mean = 54 per cent).

Norepinephrine and isoproterenol in vivo. Because of the unacceptable mortality occurring when isoproterenol was administered in a dose equal to that of epinephrine or norepinephrine (5.50 μmoles/kg), the dose of the former was reduced to half that of the latter agents. The administration of norepinephrine or of isoproterenol to rats led to increases in rates of protein synthesis in the perfused hearts of these animals that were similar to the increases produced by epinephrine administration (Table 4).

Phenelzine or iproniazid plus epinephrine in vivo. To determine whether the MAO inhibitors phenelzine and iproniazid would prevent the increase in cardiac protein synthesis provoked by the administration of epinephrine, either one or the other of these drugs was administered once per day prior to as well as during the administration of epinephrine to rats. All rats, experimental and control, were fasted from the time of first injection with a drug to the time they were killed. The MAO inhibitors were administered at 5 p.m. on the day before epinephine administration was begun and also on the following two mornings. In the experiments with iproniazid, the usual dose of epinephrine was cut in half to 2.75 μmoles/kg because the mortality of rats given iproniazid plus the full dose of epinephrine was too high. Phenelzine inhibited the rise in protein synthesis induced by epinephrine significantly, but to a small degree (31 per cent), while iproniazid had no such effect. Either

Table 4. Effect of administration of norepinephrine or isoproterenol *in vivo* on rate of protein synthesis in isolated perfused hearts

Expt.	Labeled amino acid	Perfusion time (hr)	Radioactivity of cardiac protein: Control (dis./min/mg)	Radioactivity of cardiac protein: Drug (dis./min/mg)	Radioactivity of cardiac protein: Change (%)	Incorporation of labeled amino acid into cardiac protein: Control (μmoles/g)	Incorporation of labeled amino acid into cardiac protein: Drug (μmoles/g)	Incorporation of labeled amino acid into cardiac protein: Change (%)
				Norepinephrine*				
1	[^{14}C]phe	1.00	1,064	2,137	+100.8	1.33	1.85	+38.9
2	[^{14}C]phe	1.25	1,381	1,768	+28.0			
3	[^{14}C]phe	1.50	1,597	2,108	+32.0	1.47	2.69	+83.1
4	[^{14}C]phe	2.00	2,577	3,733	+44.9	1.67	2.72	+61.6
Mean					+51.4			+61.2
± S.E.M.					±16.85			±12.76
				Isoproterenol*				
5	[^{14}C]phe	2.0	2,117	3,175	+50.0	1.89	2.81	+48.7
6	[^{14}C]phe	2.0	2,532	3,596	+42.0	2.33	3.09	+32.6
7	[^{14}C]phe	2.0	2,249	2,591	+15.2	1.78	2.43	+36.5
8	[^{3}H]leu	2.0	10,889	14,212	+30.5			
Mean					+34.4			+39.3
± S.E.M.					±7.56			±4.85

* Norepinephrine or isoproterenol, in suspension in oil, in a dose of 5.5 and 2.7 μmoles/kg, respectively, was injected s.c. three times during a period of 24 hr into experimental rats while controls were injected with oil vehicle alone. Hearts were removed from the animals 5 hr after the third injection and were perfused in the presence of a radioactive amino acid, unlabeled amino acids, and glucose.

Table 5. Effect of administration of phenelzine or iproniazid *in vivo* on increase in protein synthesis in isolated perfused rat hearts induced by administration of epinephrine *in vivo**

			Radioactivity of cardiac protein							Incorporation of labeled amino acid into cardiac protein						
							Change							Change		
Expt.	Labeled amino acid	Perfusion time (hr)	Control (dis./min/mg)	MAOI† (dis./min/mg)	Epinephrine alone (dis./min/mg)	MAOI + epinephrine (dis./min/mg)	MAOI vs control (%)	Epinephrine vs control (%)	MAOI + epinephrine vs control (%)	Control (μmoles/g)	MAOI (μmoles/g)	Epinephrine alone (μmoles/g)	MAOI + epinephrine (μmoles/g)	MAOI vs control (%)	Epinephrine vs control (%)	MAOI + epinephrine vs control (%)
								Phenelzine								
1	[^{14}C]phe	1	1025	976	1469	1191	−4.8	+43.3	+16.2	1.64	1.62	2.37	2.02	−1.2	+44.5	+23.2
2	[^{14}C]phe	1	1154	1189	1734	1506	+3.0	+50.3	+30.5	1.45	1.52	2.23	1.99	+4.8	+53.8	+37.2
3	[^{14}C]phe	1	939	944	1149	1190	+0.5	+22.4	+26.7	1.26	1.18	1.70	1.74	−6.3	+34.9	+38.1
4	[^{14}C]phe	1	1033	1078	1445	1331	+4.4	+39.9	+28.8	0.97	1.03	1.33	1.26	+6.2	+37.1	+29.9
5	[^{14}C]phe	1	1139	1201	1528	1392	+5.4	+34.2	+22.2	1.39	1.43	1.86	1.55	+2.9	+33.8	+11.5
6	[^{14}C]phe	2	1839	1873	2271	2107	+1.9	+23.5	+14.6	2.14	1.98	2.82	2.63	−7.5	+31.8	+22.9
Mean							+1.73	+35.6	+23.2					−0.18	+39.3	+27.1
± S.E.M.							±1.49	±4.53	±2.71‡					±2.36	±3.40	±4.11‡
								Iproniazid								
7	[^{14}C]phe	1	1285	1173	1679	1598	−8.7	+30.7	+24.4	1.17	1.20	1.68	1.72	+2.6	+43.6	+47.0
8	[^{14}C]phe	1	1077	1204	1523	1731	+11.8	+41.4	+60.7	1.10	1.19	1.75	2.13	+8.2	+59.1	+79.0
9	[^{14}C]phe	1	1209	1225	1533	1660	+1.3	+26.8	+37.3	1.39	1.30	1.47	1.88	−6.5	+23.5	+35.3
10	[^{3}H]leu	1	6494	6237	7794	7673	−4.0	+20.0	+18.2							
Mean							+0.10	+29.7	+35.2					+1.43	+42.1	+53.8
± S.E.M.							±4.40	±4.48	±9.40					±4.28	±10.3	±13.1

* Epinephrine, in suspension oil, in a dose of 5.5 μmoles/kg in the phenelzine experiments and of 2.7 μmoles/kg in the iproniazid experiments, was injected s.c. on 2 successive days into experimental rats while controls were injected with oil vehicle alone. Some of these experimental animals were also injected s.c. three times with 25 mg/kg of phenelzine sulfate or iproniazid phosphate. Other rats were injected with only one or the other of the two drugs, or with saline. Hearts were removed from all animals 5 hr after the last injection of epinephrine and perfused with Krebs–Ringer bicarbonate buffer solution containing a radioactive amino acid, nonradioactive amino acids, and glucose. All rats were fasted during the entire period of drug administration.

† MAOI = monoamine oxidase inhibitor.

‡ The difference between the change produced by administration of epinephrine alone and that produced by epinephrine plus phenelzine is statistically significant ($P < 0.05$).

MAO inhibitor alone did not affect the rate of protein synthesis significantly (Table 5).

DISCUSSION

The isolated perfused heart has been employed as a suitable preparation for the study of the effects of cardiac overload, oxygenation, and composition of the perfusion medium on the rate of cardiac protein synthesis [29–32]. Radioactive leucine and phenylalanine, which we used, have been reported to be particularly satisfactory as protein precursor markers in this preparation [30]. There is a rapid equilibration of these amino acids across heart cell membranes. Phenylalanine not only equilibrates very rapidly with an intracellular pool of stable size, but is not produced or converted to other amino acids during heart perfusion [30]. Furthermore, there is minimal reutilization of phenylalanine released from the heart protein [33].

We previously reported that the subcutaneous injection of one or more large doses (5.46 μmoles/kg) of epinephrine, norepinephrine or isoproterenol (L-isomers) in oil suspension, into rats, increased the rate of cardiac protein significantly *in vitro* in slices of heart left ventricle obtained from these animals, a significant rise occurring as early as 5 hr after a single injection of epinephrine. We have now found that such an enhancement of cardiac protein synthesis is also seen in isolated perfused hearts of rats treated with the same doses of these agents. The increases were somewhat smaller in our perfused hearts than they had been in heart slices, for reasons that are not apparent to us. We have observed, in a few preliminary experiments not yet reported, that injection of high doses of sympathomimetic amines into rats also increased the rate of cardiac protein synthesis when the latter was determined *in vivo*. This lends support to the assumption that the changes in rates we have observed *in vitro* truly reflect a phenomenon occurring in the intact animal.

The increased rate of protein labeling in the isolated perfused hearts after the administration *in vivo* of sympathomimetic amines was not due to increased intracellular specific acitivities of the labeled amino acids in these hearts; there were no significant differences in intracellular specific activities of radioactive amino acid precursor between control and drug-treated rat hearts, and increased rates of protein labeling were manifested when intracellular specific acitivities were taken into consideration and the rates of incorporation expressed as μmoles of precursor amino acid/g of cardiac protein/perfusion period.

The observed increase in cardiac protein synthesis could involve a number of different mechanisms. Thus, it could be caused by a direct stimulation of intracellular processes of protein synthesis by the sympathomimetic amines, by an indirect stimulation of such processes resulting from catecholamine-provoked enhancement of cardiac function, or it could be part of an adaptive and/or reparative response of the heart to the damage caused by high levels of the drugs. Our observations that addition of these agents *in vitro* to incubating heart slices [17] or to isolated perfused hearts did not produce an increase of protein synthesis argue against a direct effect. In the case of perfused hearts, addition of epinephrine actually caused small but persistent decreases in the rates of incorporation of the labeled amino acids into protein. This had not been the case with incubated slices of heart. We have no ready explanation for this difference. Our results with isolated perfused hearts, however, are more consistent with the findings of other investigators that the addition of epinephrine *in vitro* reduced the rate of incorporation of amino acids into the protein of sections of diaphragm and liver as well as of epididymal fat pads of rats [34–36].

The increased rate of protein synthesis provoked by the administration of sympathomimetic amines *in vivo* may be a consequence of increased cardiac function such as rate, contractile force or work produced by the drugs. Such an indirect stimulatory effect would have to more than compensate for any direct small inhibitory effect, if the latter also occurs in the intact animal. The fact that addition of epinephrine *in vitro* to the isolated perfused nonworking hearts caused increases in heart rates but not in protein synthesis argues against increased heart rate *per se* as being an adequate stimulus for augmentation of protein synthesis. It is well known, however, that cardiac overload results in enhanced cardiac protein synthesis [31, 37, 38] and ultimately in cardiac hypertrophy. If the sympathomimetic amines act in this manner, then they may increases protein synthesis and heart size by mechanisms no different from those responsible for these effects when the latter are provoked by aortic constriction or experimental hypertension.

Since large doses of sympathomimetic amines cause cardiac necrosis as well as hypertrophy, it is possible that the observed enhancement of cardiac protein synthesis is associated with cardiac damage rather than with increased cardiac function. Thus, an increase in protein synthesis may occur as an adaptive response of reversibly injured cells [39] to their injury. It may even be due to the activity of non-myocardial cells infiltrating necrotic areas during the inflammatory response to injury. Polymorphonuclear leucocytes, large monocytes and histiocytes accumulate in the injured zones [1, 10, 40]. The macrophages actively synthesize proteins, including lysosomal enzymes [41]. At the same time and subsequently, there is a reparative response which includes infiltration with fibroblasts which proliferate, are active in protein synthesis, and secrete collagen [10, 40, 41].

We thought that the administration of MAO inhibitors might prevent or reduce the increase in cardiac protein synthesis provoked by sympathomimetic amines if the increase was a consequence of cardiac damage, since the MAO inhibitors have been found to decrease such damage [18–20]. Our data show that the inhibition of the epinephrine-induced increase in protein synthesis was small (about 30 per cent with phenelzine) or did not occur at all (with iproniazid). Stanton and Schwartz [20] reported that the cardiomegaly (as well as necrosis), caused in rats by the subcutaneous injection of isoproterenol in aqueous solution on 2 consecutive days, could be diminished by administration of phenelzine (15–50 mg/kg) to these animals on the 2 preceding days as well as on the days of isoproterenol treatment. However, the early increases in heart weights caused by isoproterenol were found by the above authors to be mainly

due to increases in cardiac water content and therefore may not have reflected increases in rates of cardiac protein synthesis. If the two MAO inhibitors we tested may be considered representative, then these agents are not potent inhibitors of the elevations in rates of cardiac protein synthesis produced by large doses of sympathomimetic amines. Since the former drugs have been found capable of diminishing isoproterenol-induced cardiac necrosis considerably, our results may support the view that the effect of sympathomimetic amines on cardiac protein synthesis is not a consequence of or dependent upon the production of cardiac necrosis.

Acknowledgement—I would like to express my appreciation to Anne Gualtieri for her excellent technical assistance.

REFERENCES

1. G. Rona, I. Chappel, T. Balazs and R. Gaudry, *Archs Path.* **67**, 443 (1959).
2. A. A. Alousi and S. Mallov, *Am. J. Physiol.* **206**, 603 (1964).
3. R. A. Mueller and J. Axelrod, *Circulation Res.* **23**, 771 (1968).
4. H. C. Stanton, G. Brenner and E. D. Mayfield, *Am. Heart J.* **77**, 72 (1969).
5. C. I. Chappel, G. Rona, T. Balazs and R. Gaudry, *Can. J. biochem.* **37**, 35 (1959).
6. H. M. Maling, B. Highman and E. C. Thompson, *Am. J. Cardiol.* **5**, 628 (1960).
7. G. Zbinden and R. A. Moe, *Ann. N.Y. Acad. Sci.* **156**, 294 (1969).
8. V. J. Ferrans, R. G. Hibbs, H. S. Weily, D. G. Weilbaecher, J. J. Walsh and G. E. Burch, *J. molec. Cell Cardiol.* **1**, 11 (1970).
9. B. Ostadal and R. Rychterova, *Physiologia bohemoslov.* **20**, 541 (1971).
10. Z. Csapo, J. Dusek and G. Rona, *Archs Path.* **93**, 356 (1972).
11. J. E. Szakacs and A. Cannon, *Am. J. clin. Path.* **30**, 425 (1958).
12. L. K. Kline, *Am. J. Path.* **38**, 539 (1961).
13. P. O. VanVliet, H. B. Burchell and J. L. Titus, *New Engl. J. Med.* **274**, 1102 (1966).
14. A. G. Rose, *S. Afr. med. J.* **48**, 1285 (1974).
15. K. K. Gupta, *Lancet* 1, 281 (1975).
16. J. E. Szakacs and B. Mehlman, *J. Cardiol.* **5**, 619 (1960).
17. S. Mallov, *J. Pharmac. exp. Ther.* **187**, 482 (1973).
18. G. Zbinden, *Am. Heart J.* **60**, 450 (1960).
19. G. Zbinden and R. E. Bagdon, *Revue can Biol.* **22**, 357 (1963).
20. H. C. Stanton and A. Schwartz, *J. Pharmac. exp. Ther.* **157**, 649 (1967).
21. R. B. Arora and D. S. Sivappa, *Br. J. Pharmac. Chemother.* **19**, 394 (1962).
22. R. B. Arora, *Ann. N.Y. Acad. Sci.* **107**, 1152 (1963).
23. A. M. Master, *Am. Heart J.* **56**, 570 (1958).
24. H. L. Russek, *Angiology* **11**, 76 (1960).
25. W. B. Abrams, M. C. Becker, D. W. Lewis and J. H. Killough, *Am. J. Cardiol.* **5**, 634 (1960).
26. W. Hollander, A. V. Chobanian and R. W. Wilkins, *Am. J. Cardiol.* **6**, 1136 (1960).
27. D. Horwitz and A. Sjoerdsma, *Ann. N.Y. Acad. Sci.* **107**, 1033 (1963).
28. J. R. Neeley, H. Liebermeister and H. E. Morgan, *Am. J. Physiol.* **212**, 815 (1967).
29. R. Scharff and I. G. Wool, *Biochem. J.* **97**, 257 (1965).
30. H. E. Morgan, D. C. N. Earl, A. Broadus, E. B. Walpert, K. E. Giger and L. S. Jefferson, *J. biol. Chem.* **246**, 2152 (1971).
31. S. S. Schreiber, M. Oratz and M. A. Rothschild, *Am. J. Physiol.* **211**, 314 (1966).
32. S. S. Schreiber, C. Evans, M. Oratz and M. A. Rothschild, *Am. J. Physiol.* **212**, 35 (1967).
33. S. S. Schreiber, M. Oratz, I. Klein and M. A. Rothschild, in *Recent Advances in Studies on Cardiac Structure and Metabolism* (Eds. N. S. Dhalla and G. Rona), Vol. 3, pp. 589–601. University Park Press, Baltimore (1973).
34. I. G. Wool, *Am. J. Physiol.* **198**, 54 (1960).
35. M. G. Herrara and A. E. Renold, *Biochim. biophys. Acta* **44**, 165 (1960).
36. J. Pryor and J. Berthet, *Archs int. Physiol. Biochim.* **68**, 227 (1960).
37. S. Gudbjarnason, M. Telerman and R. J. Bing, *Am. J. Physiol.* **206**, 294 (1964).
38. H. A. Johnson, in *Cardiac Hypertrophy* (Ed. N. R. Alpert), pp. 21–30. Academic Press, New York (1971).
39. J. Pilny, E. Faltova and Z. Deyl, *Physiologia bohemoslov.* **20**, 549 (1971).
40. G. Rona, D. S. Kahn and C. I. Chappel, *Revue can. Biol.* **22**, 241 (1963).
41. R. J. Bing, *Cardiology* **56**, 314 (1971/72).