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Plasma free fatty acid and blood glucose responses to analogues of norepinephrine in man

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SUMMARY

L-Norepinephrine, L-epinephrine, DL-isoproterenol, and L-α-methyl norepinephrine produced pronounced elevations of plasma free fatty acid (FFA) concentrations in five normal subjects during 30-minute constant infusions at dose rates adjusted to give a pressor response of 45 ± 20 mm Hg. DL-Synephrine produced moderate increases, and tyramine produced minimal increases in FFA levels. Equipressor infusions of dopamine, L-phenylephrine, DL-normetanephrine and L-*N*-methyl epinephrine produced no significant FFA elevations. The observations suggest that, in derivatives of phenylethylamine, hydroxyl substitution at the *para* position and on the β-carbon and the presence of a primary or secondary amine structure are associated with the potent ability to elicit elevations of FFA in man. The phenolic hydroxyl group in the *meta* position appears to be of lesser, though significant, importance. Changes in blood glucose concentration did not always correlate with plasma FFA changes. During infusions of DL-isoproterenol, FFA rose significantly while glucose did not. During infusions of dopamine and L-*N*-methyl epinephrine, glucose rose significantly while FFA did not.

In 1956, Dole (1) and Gordon and Cherkes (2) noted marked elevations in plasma free fatty acid (FFA) concentrations after parenteral administration of epinephrine to human subjects. Since then, similar elevations of FFA have been observed after norepinephrine and isoproterenol infusions (3, 4). In this paper, a comparison is made of the changes in FFA and in glucose concentrations during infusions of equipressor doses of 10 structurally related sympathomimetic amines: L-norepinephrine, L-epinephrine, dopamine, DL-isoproterenol, tyramine, DL-normetanephrine, L-α-methyl norepinephrine, DL-synephrine, L-phenylephrine, and L-*N*-methyl epinephrine. Although the effects of these amines on blood glucose concentrations are well documented (5), a systematic study of their ability to mobilize FFA has not been reported.

METHODS

Nine male, nonobese, normal volunteers 20 to 24 years of age were studied. All nine subjects were on a regular diet, were free of known disease and were receiving no medications at the time of study. Each individual study was performed on a different day, after an overnight fast, with the subject lying quietly in a secluded room. In every case, the study was completed by 10:30 in the morning. A 20-gauge needle for intravenous infusion and an 18-gauge Cournand needle for withdrawing sequential blood samples were inserted in opposite arm veins. A small amount of intradermal 1% lidocaine was administered at the site of the Cournand needle. A 3-way stopcock opened both to a 0.9% saline solution and a test solution of one of the amines. Alternation of infusion solutions was effected by clamping or releasing one or the other infusion tubing.

During each study, the subject received an initial 30-minute saline infusion followed by a 30-minute infusion of the test solution administered by means of a calibrated Bowman constant infusion pump. The infusions were given at rates producing systolic blood-pressure elevations of 45 ± 20 mm Hg. Arm-cuff

TABLE 1. PLASMA FFA AND BLOOD GLUCOSE CONCENTRATIONS, AMINE DOSES, AND CHANGES IN BLOOD PRESSURE AND PULSE RATE

Drug	Patient No.	Dose	Plasma FFA −30 min	Plasma FFA 0	Plasma FFA 5 min	Plasma FFA 15 min	Plasma FFA 30 min	Blood Glucose −30 min	Blood Glucose 0	Blood Glucose 5 min	Blood Glucose 15 min	Blood Glucose 30 min	ΔB.P.*	Δpulse/min
		μg/kg/min	*mEq/l*					*mg %*						
Control	1	0	0.30	0.38	0.39	0.41	0.41	80	82	80	79	80	0/0	0
	2	0	0.33	0.36	0.34	0.30	0.32	88	90	86	88	84	1/0	−4
	3	0	0.31	0.31	0.32	0.29	0.29	92	92	87	96	92	0/0	0
	4	0	0.27	0.29	0.26	0.34	0.23	77	76	78	76	80	−1/−1	−4
	5	0	0.25	0.22	0.18	0.15	0.12	97	97	96	97	99	−4/−3	0
Norepinephrine	1	0.129	0.31	0.38	0.31	0.55	0.99	93	96	89	109	129	64/32	−13
	1	0.105	0.28	0.35	0.35	0.45	0.70	81	80	80	94	102	27/18	−24
	2	0.168	0.34	0.41	0.44	0.67	1.29	94	96	95	105	121	50/32	−8
	3	0.248	0.32	0.34	0.44	0.69	0.97	92	89	94	109	143	46/17	−20
	4	0.409	0.23	0.15	0.26	0.50	0.75	77	84	78	104	141	50/25	−17
	5	0.157	0.18	0.21	0.21	0.31	0.79	92	87	93	113	130	56/24	−15
Epinephrine	1	0.105	0.45	0.48	0.49	0.61	0.99	97	99	98	129	164	37/−33	28
	2	0.181	...	0.73	0.67	1.11	1.84	82	83	80	110	126	50/0	24
	3	0.145	0.38	0.36	0.45	0.71	1.07	93	92	96	123	168	46/−20	17
	4	0.204	0.27	0.33	0.43	0.59	1.13	88	84	90	121	173	35/−20	15
	5	0.136	0.15	0.25	0.29	0.60	0.73	94	94	105	132	168	58/0	15
Isoproterenol	1	0.023	0.20	0.31	0.32	0.35	0.61	89	94	95	91	98	29/−22	34
	2	0.030	0.52	0.61	0.79	1.10	1.33	87	92	89	87	87	38/−20	16
	3	0.029	0.34	0.35	0.41	0.53	0.75	92	90	94	89	93	48/−26	33
	4	0.036	0.25	0.24	0.32	0.56	0.57	84	86	88	90	90	26/−34	39
	5	0.025	0.22	0.22	0.16	0.29	0.50	96	97	99	98	96	40/−25	17
Dopamine	1	4.92	0.38	0.31	0.31	0.31	0.34	97	95	98	106	106	30/−7	−2
	2	6.19	0.40	0.47	0.45	0.47	0.49	94	95	99	102	108	42/5	10
	3	7.25	0.44	0.42	0.53	0.53	0.51	92	95	98	110	...	60/−3	−8
	4	4.96	0.19	0.19	0.20	0.21	0.20	79	83	84	96	95	50/5	15
	5	5.71	0.62	0.61	0.58	0.56	0.53	95	99	100	110	115	41/−12	12
Tyramine	1	0.020	0.29	0.27	0.26	0.28	0.29	93	97	97	98	98	34/−4	−14
	2	0.026	0.63	0.70	0.69	0.69	0.71	81	82	81	81	80	59/4	−5
	3	0.025	0.34	0.42	0.59	0.56	0.47	92	94	96	96	97	36/10	−5
	4	0.031	0.38	0.37	0.38	0.38	0.39	82	81	83	90	86	40/0	−9
	5	0.021	0.23	0.19	0.23	0.29	0.31	95	98	92	93	96	33/−1	−6
Phenylephrine	1	1.24	0.32	0.38	0.37	0.33	0.37	97	95	94	98	103	62/44	−16
	2	1.27	0.64	0.63	0.66	0.64	0.79	74	79	74	80	81	28/23	−14
	3	1.62	0.38	0.37	0.38	0.43	0.39	82	84	85	87	91	65/27	−24
	4	2.64	0.10	0.15	0.25	0.21	0.23	82	82	82	95	97	49/25	−33
	5	1.17	0.36	0.33	0.26	0.26	0.33	90	91	84	86	88	34/27	−16

* Calculated from the average systolic and diastolic blood pressures in the final 10 minutes of the test infusion, minus the average of the values for the ten minutes immediately preceding the test infusion.

blood pressure and radial pulse rate were determined at 2-minute intervals throughout the study. Blood samples for determination of plasma FFA and blood glucose concentrations were drawn in a dry syringe 30 minutes before and immediately before the start of the infusion (0 time) and after 5, 15, and 30 minutes of infusion. On the initial day, in all cases except one, the subject received only a saline infusion. Thereafter, on different days, the subjects received test infusions of L-norepinephrine,[1] L-epinephrine,[2] DL-isoproterenol,[3] dopamine,[4] tyramine,[5] L-phenylephrine,[6]

[1] L-Norepinephrine bitartrate (Winthrop Laboratories, New York, N. Y.).

[2] L-Epinephrine hydrochloride (Parke-Davis and Co., Detroit, Michigan).

[3] Isoproterenol hydrochloride (Winthrop Laboratories, New York, N. Y.).

[4] 3-Hydroxy-tyramine hydrochloride (California Corp. for Biochemical Research, Los Angeles, California).

[5] Tyramine hydrochloride (California Corp. for Biochemical Research, Los Angeles, California).

[6] Phenylephrine hydrochloride (Winthrop Laboratories, New York, N. Y.).

[7] DL-Normetanephrine hydrochloride (California Corp. for Biochemical Research, Los Angeles, California).

TABLE 2. PLASMA FFA AND BLOOD GLUCOSE CONCENTRATIONS, AMINE DOSES, AND CHANGES IN BLOOD PRESSURE AND PULSE RATE

Drug	Patient No.	Dose	Plasma FFA −30 min	Plasma FFA 0	Plasma FFA 5 min	Plasma FFA 15 min	Plasma FFA 30 min	Blood Glucose −30 min	Blood Glucose 0	Blood Glucose 5 min	Blood Glucose 15 min	Blood Glucose 30 min	ΔB.P.*	Δpulse/min
		μq/kg/min			*mEq/l*					*mg %*				
Control	5	0	0.25	0.22	0.18	0.15	0.12	97	97	96	97	99	−4/−3	0
	6	0	0.35	0.44	0.35	0.36	0.36	91	87	86	83	94	3/−1	1
	7	0	0.19	0.16	0.13	0.15	0.30	88	95	95	92	88	2/2	−3
	8	0	0.45	0.51	0.45	0.35	0.54	99	103	98	96	93	−1/−1	2
	9	0	0.17	0.19	0.21	0.18	0.22	97	96	95	98	99	1/5	2
Norepinephrine	5	0.157	0.18	0.21	0.21	0.31	0.79	92	87	93	113	130	56/24	−15
	6	0.203	0.23	0.23	0.23	0.38	0.68	88	91	86	105	143	38/30	−15
	7	0.247	0.51	0.37	0.51	0.97	1.32	87	90	89	124	150	47/26	−4
	8	0.160	0.35	0.35	0.36	1.01	1.44	134	104	98	123	138	44/27	−14
	9	0.250	0.16	0.12	0.10	0.47	0.79	79	79	77	98	138	59/26	−13
α-Methyl norepinephrine	5	0.714	0.30	0.37	0.57	1.38	1.88	97	90	108	139	184	59/1	13
	6	0.580	0.37	0.45	0.40	0.94	1.14	92	91	88	119	170	25/4	0
	7	0.684	0.27	0.50	0.54	1.26	1.53	86	91	91	122	164	45/−6	12
	8	0.467	0.48	0.44	0.49	1.39	1.84	105	109	109	129	161	44/−9	4
	9	0.694	0.18	0.10	0.19	0.62	1.36	77	78	84	108	164	44/−9	5
Synephrine	5	34.3	0.19	0.23	0.19	0.30	0.34	94	99	98	104	108	43/5	−3
	6	39.1	0.20	0.31	0.20	0.25	0.57	93	93	92	104	118	61/14	−8
	7	37.0	0.47	0.51	0.40	0.66	0.73	92	92	89	99	99	48/13	0
	8	32.0	0.21	0.25	0.51	0.41	0.72	99	99	97	98	105	57/10	−2
	9	34.7	0.30	0.35	0.34	0.55	0.95	96	101	96	106	111	38/−10	−1
Normetanephrine	5	171	0.23	0.30	0.33	0.30	0.52	88	94	91	95	99	37/28	−15
	6	188	0.51	0.27	0.41	0.33	0.30	96	98	95	108	122	36/34	−12
	7	226	0.42	0.42	0.42	0.39	0.60	91	91	91	98	94	48/31	−13
	8	173	0.35	0.48	0.37	0.54	0.51	100	101	99	107	108	29/23	−8
	9	208	0.21	0.26	0.08	0.16	0.16	88	87	87	95	97	48/25	−15
N-methyl epinephrine	5	5.71	0.34	0.33	0.36	0.40	0.51	84	88	83	98	115	41/27	−11
	7	6.16	0.39	0.36	0.37	0.37	0.48	85	82	82	95	107	29/32	−8
	9	6.94	0.34	0.36	0.31	0.30	0.33	78	82	84	93	119	42/13	−14

* Calculated from the average systolic and diastolic blood pressures in the final 10 minutes of the test infusion, minus the average of the values for the ten minutes immediately preceding the test infusion.

DL-synephrine, L-α-methyl norepinephrine, DL-normetanephrine,[7] or L-*N*-methyl epinephrine administered in a 0.9% solution of sodium chloride in varying sequence. The dose of each of the amines was expressed in terms of the base.

Heparinized blood samples for FFA determinations were stored in crushed ice. The plasma was extracted within 3 hours after collection. FFA concentrations were determined by extraction in a mixture of 2,2,4-trimethylpentane (isooctane), glacial acetic acid, and acetic anhydride, followed by titration of the isooctane phase with 0.02 N NaOH after two washings (6).

Blood-glucose concentrations were determined by the Hoffman ferricyanide method, using the modification of Skeggs for use with the autoanalyzer (7).[8]

[8] The authors are indebted to the Central Chemical Laboratory of the Clinical Center, National Institutes of Health, for the blood glucose determinations.

Statistical significance was determined by the standard "*t*" test with two-tailed distribution. In the calculations, the changes in FFA and glucose levels occurring between 0 time and 30 minutes of amine infusions were compared with changes occurring during saline infusions.

RESULTS AND DISCUSSION

The effects of the amine infusions on FFA levels, glucose levels, blood pressure, and heart rate are presented in Tables 1 and 2. Figure 1 shows graphically the relative changes in FFA and glucose levels after 30-minute equipressor infusions.

Alpha-methyl norepinephrine caused the greatest increases, both in FFA and blood glucose concentrations. Norepinephrine, epinephrine, and isoproterenol follow in relative FFA-mobilizing activity. Synephrine had moderate ability to mobilize FFA ($p < 0.02$) and tyr-

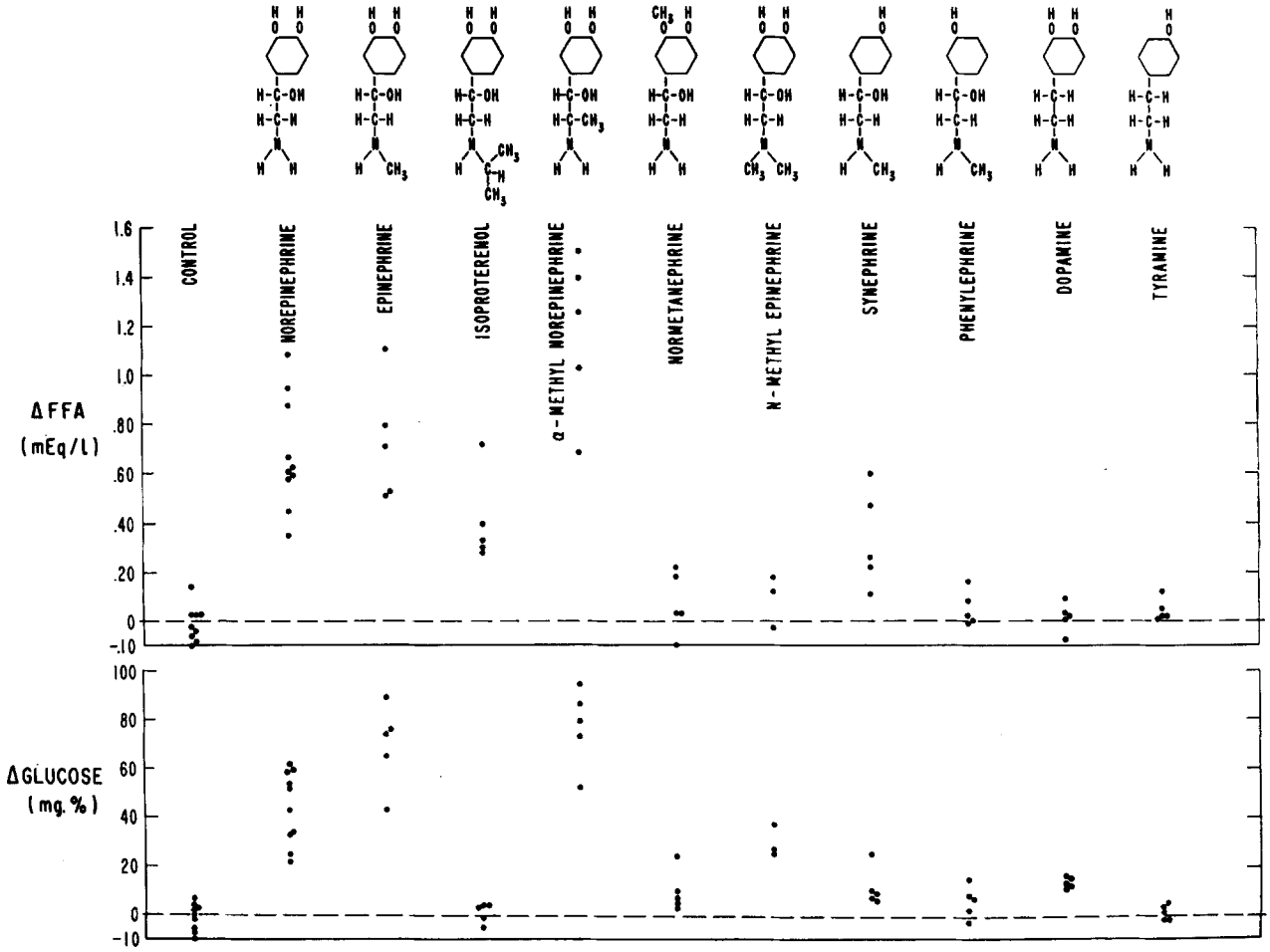

FIG. 1. Summary of the changes in plasma FFA and blood glucose levels after 30-minute infusions of normal saline and the 10 amines (data from Tables 1 and 2.) (The blood glucose increment after 15 minutes of dopamine infusion was plotted for patient No. 3 in the absence of a 30-minute sample.)

amine had a very small but significant effect on FFA ($p < 0.05$). Dopamine, phenylephrine, normetanephrine and *N*-methyl epinephrine had no significant effect on FFA ($p > 0.05$) in the dose ranges used. No consistent difference in effect on FFA was noted between those amines with predominant constrictor and those with predominant dilator effects on blood vessels.

From these observations, by comparing derivatives of phenylethylamine used at equipressor doses, one can define the amine structures associated with the fatty acid effect. Greatest activity occurs with primary and secondary catecholamines, which are hydroxylated on the β carbon of the side chain. Effects on FFA levels are preserved after substitution on the α-carbon of the side chain and after lengthening the chain substituted on the amine group. They are strikingly decreased, however, after loss of the β-hydroxyl group or formation of a tertiary amine. Removal of the phenolic hydroxyl group at the *para* position causes a greater loss of potency than does removal of the *meta* hydroxyl group. Methylation of the *meta* hydroxyl group strikingly quenches activity.

Increases in glucose were not always associated with FFA increases and *vice versa*. Isoproterenol infusions produced significant rises in FFA ($P < 0.001$) but none in glucose ($p > 0.05$). A similar effect was recently noted by Bruce and his associates in cardiac patients (4). Dopamine, on the other hand, significantly increased glucose ($p < 0.001$) but not FFA ($p > 0.05$). *N*-Methyl epinephrine resembled dopamine in its effects on glucose and FFA, but conclusions were limited by the small number of observations. The effects of catecholamines on glucose and fatty acids would appear from these data to be dissociable. Relatively different magnitudes of change in blood glucose and FFA

concentrations in response to norepinephrine and epinephrine infusions have been previously noted in dogs (8) and in man (3). In addition, Havel and Goldfien (3) noted that dibenamine blocked the effect of epinephrine on plasma FFA concentrations without altering the rise in glucose levels.

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