Effect of sympathomimetic compounds with β-adrenergic effects on plasma free fatty acids in man

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ABSTRACT Nine sympathomimetic compounds were infused intravenously in man, and their effects on the level of plasma free fatty acids, pulse rate, and blood pressure were recorded. Each compound raised the level of plasma free fatty acids. Its activity in this respect could be correlated with its activity in producing β-adrenergic effects on the circulation; both effects were abolished by pretreatment with propranolol. It is concluded that sympathomimetic compounds that stimulate β-receptors will raise the plasma concentration of free fatty acids.

KEY WORDS sympathomimetic compounds · intravenous · plasma · free fatty acids · man · α- and β-adrenergic · effects · blockade · phenoxybenzamine · propranolol

Mueller and Horwitz have shown (1) that intravenous infusions of norepinephrine, epinephrine, isoproterenol, and α-methyl norepinephrine in man produced large elevations in the concentration of free fatty acids (FFA) in plasma. The dose of the amines was adjusted to cause a rise of blood pressure of about 45 mm of Hg; in doses producing similar effects on blood pressure *dl*-synephrine produced moderate, and tyramine very small increases of FFA, but dopamine, *l*-phenylephrine, *dl*-normetanephrine, and dimethyl norepinephrine were ineffective. Bogdonoff, Linhart, Klein, and Estes (2) could demonstrate rises of plasma FFA only in response to epinephrine, norepinephrine, and isoproterenol, and suggested that release of FFA was dependent on a certain molecular configuration.

Pilkington, Lowe, Robinson, and Titterington showed (3) that in man the rise of plasma FFA due to epinephrine was prevented by β-adrenergic but not by α-adrenergic blockade; it seems therefore to be mediated by β-receptors (4). Sympathomimetic compounds should, then, be compared on the basis of their relative potency in stimulating β-receptors and not on the basis of their effect in raising mean blood pressure (which is largely due to α-adrenergic effects on the peripheral resistance); the dose should be adjusted to produce detectable β-adrenergic effects (e.g., rise of pulse rate and pulse pressure, stimulation of respiration, and increase of forearm blood flow).

In this paper we report experiments which show that infusion of various sympathomimetic compounds elicits a rise of plasma FFA which is correlated with the rise of pulse rate.

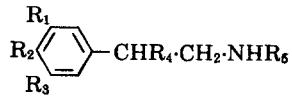
METHODS

Forty-four experiments were carried out in six normal subjects after an overnight fast. After the subject had rested for 30 min an intravenous infusion via a forearm vein was begun. Saline was infused for 30 min, then the test compound for 30 min, and finally saline for 30 min. Blood samples (10 ml) were taken every 15 min from a suitable vein in the opposite forearm (using the same site for each sample). Pulse rate was recorded twice between each blood sampling, and in some experiments blood pressure was also recorded by sphygomanometer. The blood samples were immediately put into tubes containing dried lithium heparin (100 IU) and centrifuged; plasma FFA concentration was estimated by the semiautomated colorimetric procedure of Antonis (5).

Adrenergic blocking drugs were given in the following dosage: phenoxybenzamine, 10 mg orally, five times

Abbreviation: FFA, free fatty acids.

TABLE 1 CHEMICAL COMPOSITION AND DOSES OF COMPOUNDS INFUSED



R_1	R_2	R_3	R_4	R_5	Name	Dose	No. of Expts.
OH	OH	H	OH	$CH(CH_3)_2$	*dl*-Isoproterenol	2–4 μg/min	4
OH	OH	H	OH	H	*l*-Norepinephrine	10–40 μg/min	4
OH	H	OH	OH	$CH(CH_3)_2$	*dl*-Orciprenaline	50 μg/min	5
OH	OH	H	OH	$CH(CH_3)_2$	*d*-Isoproterenol	200 μg/min	2
H	OH	H	OH	$CH(CH_3)_2$	*dl*-M.I.39	500 μg/min	5
OH	OH	H	H	CH_3	Epinine	400–800 μg/min	7
OH	OH	H	H	$CH(CH_3)_2$	WIN 5571	200–900 μg/min	4
OH	OH	H	H	H	Dopamine	0.5–3 mg/min	8
H	OH	H	OH	C_4H_9	*dl*-Bamethan	3–7 mg/min	5

daily for 2 days beforehand, followed by 10 mg intravenously at the start of the infusion of saline; propranolol, 20 mg orally 2 hr and 1 hr before the saline infusion, and a further 10 mg intravenously at the start of the infusion.

RESULTS

Table 1 shows the structural formulae of the compounds used and their rates of infusion. All the compounds caused a rise in plasma free fatty acid concentration. Control infusions of saline alone, with and without pretreatment with propranolol, had no effect.

Compounds with No Detectable α-Adrenergic Effects

Of the compounds illustrated in Table 1, *dl*-isoproterenol, *dl*-orciprenaline, *d*-isoproterenol, *dl*-M.I.39, WIN 5571, and *dl*-bamethan have no detectable α-adrenergic cardiovascular effects when used in man at these doses (see below). All six compounds caused increases of pulse rate and of plasma FFA which were reduced or abolished by prior treatment with the β-adrenergic blocking agent propranolol (6). Results in a typical experiment are illustrated in Fig. 1. In Fig. 2 the rise of plasma FFA is plotted against the rise of pulse rate for all the experiments, showing the correlation between the two and the reduction of both by β-adrenergic blockade.

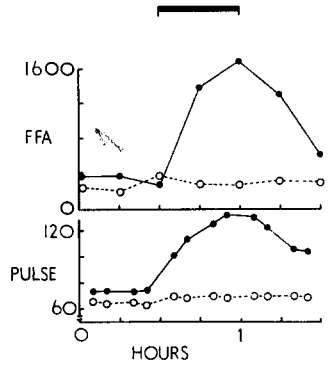


FIG. 1. The effect of infusing isoproterenol (4μg/min) during period marked ⌐¬ on the concentration (μeq/liter) of plasma FFA and pulse rate. ●——● without blocking agent. O- - -O after pretreatment with the β-adrenergic blocking agent propranolol.

Compounds with Mixed α- and β-Adrenergic Effects

Three compounds, norepinephrine (7, 8), dopamine (9), and epinine (10), in the doses used by us, have both α- and β-adrenergic effects on the human circulation. These compounds all raised plasma FFA levels; the rise

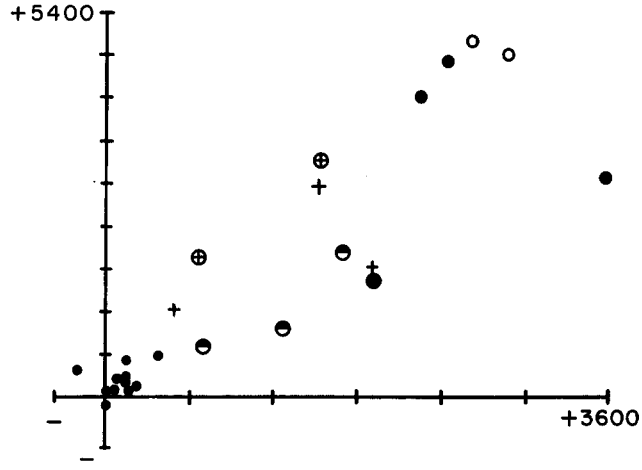


FIG. 2. Correlation between rise in plasma FFA and pulse rate caused by infusion of drugs with β- but without α-adrenergic effects. The changes have been measured by plotting the results graphically and measuring the areas under the curves obtained.
Abscissa: integral of change of pulse rate from t = 30 min (start of infusion) to t = 90 min. Ordinate: integral of change of FFA (μeq/100 ml) from t = 30 to t = 90 min.
O *dl*-M.I.39, ● orciprenaline, ⊕ *dl*-isoproterenol, ◓ *d*-isoproterenol, + WIN 5571, ◒ *dl*-bamethan; ● (close to origin) all experiments using these drugs after pretreatment with β-blocking agent propranolol.

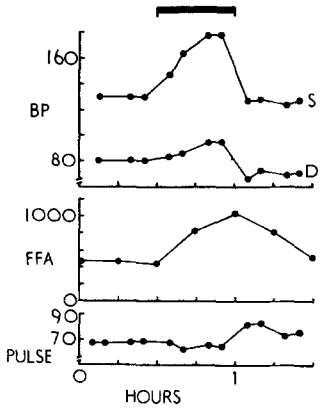


FIG. 3. The effect of infusing norepinephrine (10 μg/min) during period marked ⌐¬ on blood pressure (mm Hg, S = systolic, D = diastolic), plasma FFA (μeq/liter) and pulse rate.

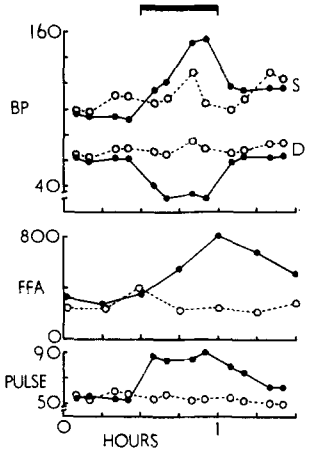


FIG. 4. The effect of infusing epinine (800 μg/min) during period marked ⌐¬ on blood pressure (mm Hg), plasma FFA (μeq/liter), and pulse rate. ●——● after pretreatment with α-adrenergic blocking agent phenoxybenzamine. O- - -O after pretreatment with above and β-adrenergic blocking agent propranolol.

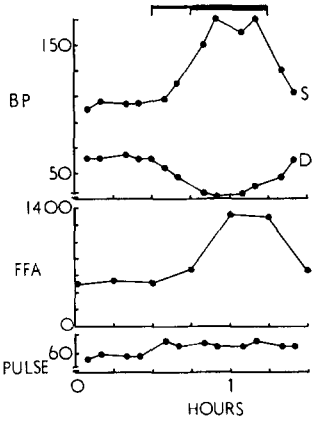


FIG. 5. The effect of infusing dopamine (0.5 mg/min for 15 min, and then 1 mg/min for 30 min, during the periods marked) on blood pressure (mm Hg), plasma FFA (μeq/liter), and pulse rate. The subject was pretreated with phenoxybenzamine.

was associated with slowing or little change in pulse rate, and a rise of arterial pressure. Results of a typical experiment with norepinephrine are illustrated in Fig. 3. With dopamine and epinine the rise of plasma FFA was barely significant and the marked rise of blood pressure deterred us from using a larger dose. After pretreatment with the α-adrenergic blocking agent phenoxybenzamine all three compounds caused a greater rise of plasma FFA (Table 2); epinine now caused a rise of pulse rate, and both pulse rate and plasma FFA changes were prevented by pretreatment with propranolol in addition to phenoxybenzamine (Fig. 4). After phenoxybenzamine both dopamine and norepinephrine failed to increase the pulse rate even though they caused a large increase of blood pressure (a typical experiment with dopamine is illustrated in Fig. 5); both rise of FFA and rise of pulse pressure were prevented by propranolol. However, without phenoxybenzamine, propranolol did not prevent the moderate rise in plasma FFA due to dopamine (Table 2).

DISCUSSION

The experiments with *dl*-isoproterenol, *dl*-orciprenaline, *d*-isoproterenol, *dl*-M.I.39, WIN 5571, and *dl*-bamethan are relatively easy to interpret. These compounds were selected as having no detectable α-adrenergic effects on the circulation in the dosage used; i.e., on intravenous infusion they did not raise mean arterial pressure, or reduce hand blood flow and after β-adrenergic blockade

TABLE 2 INTEGRAL OF THE CHANGES OF PLASMA FFA CAUSED BY EPININE, DOPAMINE, AND NOREPINEPHRINE, AND THE EFFECTS OF ADRENERGIC BLOCKADE

	Infusion Rate	Subject	None	Pretreatment		
				Propranolol	Phenoxybenzamine	Propranolol + phenoxybenzamine
	mg/min				*μeq/100 ml*	
Dopamine	1	R. F.	1090	1035	3202	480
	0.5	T. P.	1380	1320	2580	810
Norepinephrine	0.03	G. W.	2550	80	3300	195
Epinine	0.8	R. F.	1680	—	2610	90
	0.4	T. P.	1830	230	3060	360

Values are the sum of changes in FFA level from t = 30 to t = 90 min.

of the forearm they did not reduce forearm blood flow (R. D. Lowe and B. F. Robinson, unpublished observations).

The activity of these compounds in raising plasma FFA correlates with their activity in increasing the pulse rate and both actions are prevented by β-adrenergic blockade; the order of potency of these compounds is the same for the effect on pulse rate as for that on plasma FFA. These results suggest strongly that both actions are mediated by β-receptors. Activity in raising plasma FFA is not confined to compounds with the chemical structure outlined by Bogdonoff et al. (2); rather, this chemical structure is necessary only for maximum activity.

The experiments with epinine, dopamine, and norepinephrine illustrate the difficulties of interpretation that arise when both α- and β-adrenergic effects occur simultaneously. In addition to direct inotropic and chronotropic effects on heart muscle, which are β-adrenergic effects, norepinephrine, epinine, and dopamine have peripheral vasoconstrictor effects mediated by α-receptors. The inotropic effect causes an increase of pulse pressure but the peripheral constrictor effects (potentiated by the inotropic effect) cause an increase of mean blood pressure and reflex slowing of the heart rate. The rise of blood pressure limits the dose that can be reasonably given to volunteer subjects, and the reflex slowing of heart rate masks the direct chronotropic effects; activity of each compound in raising plasma FFA and correlation with other β-adrenergic effects may therefore be overlooked. In the case of epinine, both these effects are uncovered after blockade of α-receptors and prevented by additional blockade of β-receptors. With dopamine and norepinephrine there was still little or no rise of pulse rate even after blockade of the α-receptors, probably because the blockade was incomplete.

The finding that phenoxybenzamine potentiated the rise of plasma FFA due to dopamine and epinine was unexpected. The two most likely explanations of this finding are that phenoxybenzamine either interferes with inactivation of the infused compounds or prevents their uptake into tissue stores. Whatever the explanation, the potentiation is further evidence that the rise of plasma FFA is not mediated by α-receptors.

Other workers have provided evidence that α-adrenergic blocking drugs may interfere with release of FFA from rat adipose tissue (10–12). There are considerable species differences in mobilization of FFA from adipose tissue (13), and, in addition, these experiments differ from ours in the concentration of drugs used. Wenke, Mühlbachová, and coworkers (11, 12) could detect no inhibition of catecholamine-induced release of FFA, except with concentrations of phentolamine of 4×10^{-4} M and higher; significant α-adrenergic blocking effects can be produced in man with a dose of phentolamine of about 0.1 mg/kg, representing a concentration in the range 10^{-6} to 10^{-7} M. Schotz and Page (10) used phentolamine in a dose of 15 mg/kg, and phenoxybenzamine in a dose of 8 mg/kg in vivo; in vitro the phentolamine concentration was 5×10^{-4} M or higher. At high concentrations these compounds may well have either true β-adrenergic blocking effects or nonspecific toxic effects.

The effect of dopamine in our experiments differed from the effect of the other compounds. The small rise of plasma FFA due to dopamine infusion was not abolished by propranalol. When dopamine was infused after administration of phenoxybenzamine there was a large increase of plasma FFA and this large increase did not occur after administration of both phenoxybenzamine and propranolol. The simplest hypothesis to explain these results is that dopamine has a direct effect on plasma FFA levels, is not mediated by adrenergic receptors, and in addition has an indirect effect mediated by β-receptors, perhaps after conversion to norepinephrine; the latter effect is potentiated by phenoxybenzamine.

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