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In Vivo and In Vitro Manifestations of Adrenergic Blockade in *Bordetella pertussis*-vaccinated Mice[1]

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The in vivo metabolic events which follow the administration of epinephrine, norepinephrine, or isoproterenol were examined in normal, *Bordetella pertussis*-vaccinated, and α and β adrenergically blocked mice. The normal hyperglycemic response to epinephrine was suppressed in all experimental groups. The pertussis-sensitized and β-blocked animals produced similar split patterns of altered response not duplicated by the α-blocking compounds. Those catecholamines that normally increase free fatty acids and lactic acid in the circulation failed to do so in the pertussis-sensitized and β-blocked animals; the inhibition of free fatty acid mobilization was also demonstrated with adipose tissue incubated in vitro. An extract of the pertussis organism added to incubation media prevented the catecholamine-induced free fatty acid response. The epinephrine-stabilizing effect of bovine serum albumin (Cohn-fraction V) was observed. The results of these studies further emphasize a correlation between pertussis-sensitized and β-adrenergically blocked mice.

It is well established that certain strains of rats and mice become extremely sensitive to histamine, serotonin, and other stimuli (13, 15) after the injection of *Bordetella pertussis* organisms. Several hypotheses have been proposed to explain the basic mechanism underlying the histamine and serotonin hypersensitivities (20). Of particular interest to us was the possibility that the pertussis organism, directly or indirectly, produced a malfunction in the autonomic nervous system (9). This idea was based primarily upon the failure of epinephrine to induce the normal hyperglycemic response in either pertussis-sensitized or β adrenergically blocked mice (8). Furthermore, pertussis-induced hypersensitivity could be duplicated by blocking the β-adrenergic receptors (9, 10). The hypothesis of β-adrenergic blockade has been supported by other observations (21) and in addition has been offered to explain pertussis-mediated phenomena such as peptone shock (25), endotoxin sensitivity (26), and anaphylaxis (29); it may even be significant in human atopic disease (4, 24).

The aim of the present study was to examine more extensively the influence of pertussis sensitization on various metabolic events controlled by the autonomic nervous system (1, 12), to attempt characterization of the adrenergic receptor(s) at one site of apparent malfunction (adipose tissue), and to investigate the influence of an extract from the pertussis organism on receptor function. The results support the concept of the existence of β-adrenergic blockade in the pertussis-sensitized mouse and point to the direct involvement of a bacterial component in producing the malfunction.

[1] Part of the material in this paper was taken from a thesis submitted by the senior author to the Graduate School of the University of Louisville in partial fulfillment of the requirements for the Ph.D. degree.

MATERIALS AND METHODS

Mice. Female, CFW, white mice obtained from Carworth Farms were employed in the present study. They were housed in groups not exceeding 50 in number and were allowed food and water ad libitum. At the time of the experiments, the animals weighed between 18 and 20 g.

Pertussis vaccine and extract. Fluid vaccine, a suspension of killed *B. pertussis* organisms, lot BP-0317, was supplied by Eli Lilly & Co., Indianapolis, Ind. This suspension was diluted 1:2 in sterile, nonpyrogenic, physiological saline. To establish the sensitive state, the animals were injected intraperitoneally with 0.5 ml of this suspension and were used in the experiments 5 days later.

A soluble extract of pertussis organisms containing the histamine sensitizing factor was kindly furnished by J. Munoz, Public Health Service, Hamilton, Mont.

Drugs. The following materials were purchased commercially from Winthrop Laboratories, New York, N.Y.: epinephrine, synthetic, as bitratrate (Suprarenin) containing 1 mg of base per ml, and isoproterenol HCl (Isuprel) containing 0.2 mg of

base per ml, lot 044YK22. Phentolamine methanesulfonate (Regitine), lot C-128123, was supplied by the Ciba Pharmaceutical Products, Inc., Summit, N.J., and Nethalide (Alderlin), lot 38174, by Imperial Chemical Industries, Ltd., Winslow Cheshire, Great Britain. Dibenamine HCl, lot 00870, and dichloroisoproterenol (DCl), lot 20522, were obtained from the Eli Lilly & Co., Indianapolis, Ind.; dl-epinephrine 14C-7 acetate (36.6 mc/mmole) and bovine serum albumin (fraction V), lot 44987, were obtained from the California Corporation for Biochemical Research, Los Angeles, Calif. Propranolol (Inderal) was kindly supplied by the Ayerst Laboratories, New York, N.Y.

In vivo experiments. With normal mice as a prototype, the effect of catecholamine administration was tested in a group of 40 animals. After overnight fast, 10 animals were injected intraperitoneally with saline, 10 with 0.3 mg of epinephrine per kg, 10 with the same dose of norepinephrine, and 10 with 0.5 mg of isoproterenol per kg. The volume (0.2 ml) was constant; 30 min later, the animals were lightly anaesthetized with ether. Blood was obtained from the subclavian artery with 0.2 ml used for glucose (22) and lactic acid (3) determinations; the remainder was added to a pool for free fatty acid (FFA) analysis (6). A portion of the liver was then removed and frozen immediately in a dry ice-alcohol mixture for subsequent measurement of glycogen content (11, 19).

The identical procedure, as described above, was followed with groups of mice that (i) had received pertussis vaccine 5 days previously, (ii) had been injected subcutaneously with 20 mg of propranolol per kg 1 hr before, and (iii) had received 1 hr before the subcutaneous administration of 10 mg of phentolamine per kg.

In vitro experiments. Paired right and left segments of peritoneal adipose tissue were excised from mice after cervical dislocation. The samples were placed immediately and maintained in cold saline until sufficient tissue was available for the experiment. Modified Ringers-bicarbonate medium, described by Love et al. (16), without glucose was used for incubation purposes. Prior to use, 5% bovine serum albumin (fraction V) was added to the above basal medium and oxygenated in an ice bath for at least 5 min.

In preparation for incubation, the paired specimens were removed from the cold saline, blotted lightly, and weighed on a Roller-Smith torsion balance. Each member of the pair was placed in a 25-ml Erlenmeyer flask containing 2.5 ml of the incubation medium. Thus, one segment served as experimental, the other as control. All test agents, such as the catecholamines and blocking compounds, were diluted to the desired concentration in a volume of 0.05 ml of basal incubation medium. All incubations were conducted at 37 C in a Dubnoff metabolic shaking incubator under a gas phase of 95% oxygen-5% carbon dioxide. The total FFA released into the incubation medium was measured by employing the same technique as that used for determining plasma FFA.

Segments of intact tissues were removed from the incubation flasks, were lightly rinsed in saline, and were homogenized in glass tissue grinders containing 3 ml of heptone-alcohol extracting solution with subsequent determination of FFA by use of the plasma method.

In all in vitro FFA determinations, the values were expressed as microequivalents of FFA released per gram of adipose tissue.

Radioactivity measurements. To determine the persistence of epinephrine activity throughout the 2-hr incubation periods used in these experiments, flasks containing complete incubation medium, a mixture of unlabeled epinephrine and ^{14}C-epinephrine (0.5 μc per ml of medium) were incubated at 37 C under oxygen phase for periods up to 6 hr. Control flasks contained the basal medium without albumin. Samples of equal volume were taken at various time intervals, were applied to Whatman no. 1 paper, and were chromatographed in butanol-acetic acid-water (4:1:1). After drying, the strips were analyzed in a model 7200 Packard Radiochromatogram Scanner with time constant, collimator settings, and range the same in all instances. Control ^{14}C-epinephrine migrated as a peak which was assigned an Ra value of 1. Since the parameters were constant in all experiments, results were expressed as units of radioactivity.

RESULTS

In vivo metabolic effects of catecholamines. The activities of epinephrine, norepinephrine, and isoproterenol as manifested by changes in the blood glucose, lactic acid, and FFA concentrations and in hepatic glycogen content of normal, pertussis-sensitized, and adrenergically blocked animals are shown in Table 1. Within 30 min of the intraperitoneal administration of epinephrine, the glucose, lactic acid, and FFA levels were elevated in the blood of control animals, accompanied by a marked decrease in liver glycogen. The latter also occurred in the normal group after the injection of norepinephrine, although no obvious changes in the blood levels of glucose, lactic acid, or FFA were observed. Administration of isoproterenol failed to induce either hyperglycemia or hepatic glycogenolysis, but the blood levels of both lactic acid and FFA were elevated.

In all of the experimental groups, epinephrine failed to elicit the hyperglycemic response. Exposure of pertussis-sensitized or β adrenergically blocked (propranolol) animals to epinephrine or norepinephrine was followed by the normal decrease in liver glycogen; this was not shown in the α adrenergically blocked (phentolamine) group. The latter were, however, normally reactive in terms of elevated lactic acid and FFA levels with what appeared to be an enhancement of the norepinephrine-induced FFA response. The pertussis-sensitized and the β-blocked groups failed

TABLE 1.

Blood glucose, lactic acid, liver glycogen, and FFA levels of normal, pertussis-sensitized, and adrenergically blocked mice after the injection of various catecholamines

Group[a]	Amine	Dose (mg/kg)	Blood glucose ± SE (mg/100 ml)	Lactic acid ± SE (mg/100 ml)	Liver glycogen ± SE (mg/100 ml)	FFA
Normal	Saline	—	94.3 ± 4.9	28.31 ± 1.87	0.30 ± 0.04	1,410.4
	Epinephrine	0.3	172.3 ± 13.9	57.34 ± 4.10	0.07 ± 0.02	2,051.3
	Norepinephrine	0.3	106.8 ± 10.1	28.89 ± 3.36	0.074 ± 0.027	1,433.4
	Isoproterenol	0.5	76.8 ± 8.4	48.04 ± 5.70	0.201 ± 0.036	1,769.1
Pertussis-sensitized	Saline	—	74.3 ± 9.07	29.71 ± 1.47	0.334 ± 0.091	1,190.3
	Epinephrine	0.3	77.3 ± 4.82	32.50 ± 3.97	0.062 ± 0.018	1,273.3
	Norepinephrine	0.3	76.1 ± 6.60	28.12 ± 3.55	0.068 ± 0.024	1,401.4
	Isoproterenol	0.5	77.1 ± 5.07	35.46 ± 4.16	0.239 ± 0.021	1,326.2
Propranolol (20 mg/kg)	Saline	—	89.6 ± 10.43	13.24 ± 1.85	0.324 ± 0.084	1,202.1
	Epinephrine	0.3	94.5 ± 3.70	14.34 ± 0.91	0.036 ± 0.012	1,319.4
	Norepinephrine	0.3	98.5 ± 4.65	13.21 ± 1.56	0.027 ± 0.003	1,421.3
	Isoproterenol	0.5	97.4 ± 5.75	13.73 ± 1.72	0.276 ± 0.034	1,361.1
Phentolamine (10 mg/kg)	Saline	—	82.3 ± 4.87	14.86 ± 1.47	0.371 ± 0.103	1,140.8
	Epinephrine	0.3	92.4 ± 5.54	38.84 ± 3.49	0.226 ± 0.096	1,926.6
	Norepinephrine	0.3	81.8 ± 6.06	15.62 ± 1.86	0.548 ± 0.177	1,851.8
	Isoproterenol	0.5	75.5 ± 5.35	29.87 ± 2.94	0.497 ± 0.104	1,604.3

[a] Ten animals per group.

[b] Values represent a single determination on blood pooled from 10 animals and are expressed in microequivalents per liter.

to respond to the normally active catecholamines with respect to increased FFA; the lacticacidemia was also greatly suppressed, particularly in the propranolol-treated animals.

In vitro effect of catecholamines on FFA release. The effects of varying concentrations of the three catecholamines, epinephrine, norepinephrine, and isoproterenol, on the release of FFA from incubated mouse adipose tissue are summarized in Fig. 1. In terms of FFA release, the maximal response was essentially the same with all the drugs tested. The doses of the catecholamines required to produce one-half maximal response (ED_{50}) differed significantly, indicating a relative order of potency of isoproterenol > norepinephrine > epinephrine. Isoproterenol, a β agonist (12), had a minimal effective dose (MED) of 2×10^{-7} M, i.e., the minimal concentration of drug which shows a statistically significant effect. Increased drug concentrations resulted in concomitant increases in medium FFA, with 9.5×10^{-6} M producing a maximal response of 38.7 ± 5.6 μeq/g of tissue. Norepinephrine, with primary action of α-receptors (12), produced a significant increase in medium FFA at a concentration of 2×10^{-6} M. With increasing doses to 10^{-5} M, medium FFA increased to a maximum of 42 ± 5.4 μeq/g of tissue. Epinephrine, a dual agonist for both α- and β-receptors (12), showed a MED

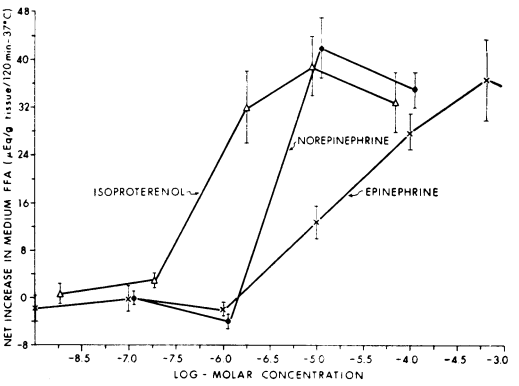

FIG. 1. *Effect of varying concentrations of catecholamines on FFA release from mouse adipose tissue in vitro. Each point represents the mean (±SE) of five experiments.*

of 4×10^{-6} M with a progressive increase in medium FFA up to 37.1 ± 8.0 μeq/g of tissue at a drug dose of 8×10^{-4} M.

In vitro effect of α- and β-adrenergic blocking agents on catecholamine-induced lipolysis. In the first portion of these studies, tissues were preincubated in various concentrations of either propranolol or dibenamine for 15 min; isoproterenol (9×10^{-6} M) was added, and incubation

continued for an additional 105 min. The FFA release occurring under these conditions is shown in Fig. 2. Propranolol appeared to be more effective in suppressing the isoproterenol-induced response than was dibenamine. At 10^{-5} M concentration, dibenamine was ineffective, whereas propranolol (10^{-5} M) suppressed approximately 35% of the normal release. Furthermore, to suppress the release by 50%, a 10^{-3} M concentration of dibenamine was required, whereas the same percentage of inhibition was observed with propranolol at a much lower concentration (4×10^{-5} M).

The second portion of these experiments involved the testing of single concentrations of α-blocking (dibenamine) and β-blocking (DCI and propranolol) agents for their influence on FFA response to epinephrine, norepinephrine, and isoproterenol. The results obtained with β-blockers are shown in Table 2. Both DCI and propranolol at the concentrations used completely suppressed the extra- and intracellular FFA response to all the catecholamines. This is in contrast to the effects of α blockers as shown in Table 3. Although suppression did occur, the degree of inhibition (65%) was not as great as with the β blockers.

Effect of fraction V on the in vitro epinephrine response of adipose tissue. Although the incubation periods employed in these studies (2 hr) are not as long as those used by some investigators (27), it was a point of concern whether epinephrine (and other catecholamines) remain active for this period of time. To test this point, epinephrine at 1.8×10^{-4} M concentration was incubated for 6 hr in media with and without the addition of 5% fraction V. At the end of the 6-hr period, samples were tested for production of hyperglycemia in normal mice. In addition, mouse

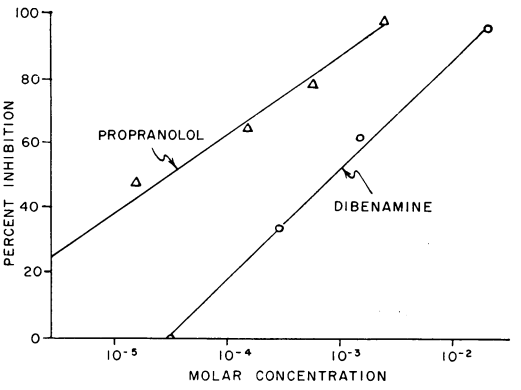

FIG. 2. *Inhibitory effect of varying concentrations of α- and β-adrenergic blocking agents on the in vitro lipolysis of mouse adipose tissue with isoproterenol (9×10^{-6} M). Per cent inhibition computed from medium FFA values of controls which contained tissue and isoproterenol, and FFA values of the experimental groups, in which the tissues were preincubated with blocking agents prior to the addition of isoproterenol.*

TABLE 2.

Effect of β-adrenergic blocking agents on catecholamine-induced lipolysis

Agent	Molar concn	Net increase in FFA[a]		
		In medium[b]	P[c]	In tissue
None	0	0		0
Epinephrine	1.0×10^{-5}	+13.2 ± 2.8	<0.01	+ 3.1
Norepinephrine	1.1×10^{-5}	+42.0 ± 5.9	<0.01	+11.9
Isoproterenol	1.8×10^{-6}	+32.0 ± 6.0	<0.01	+ 2.0
Dichloroisoproterenol (DCl)	8.8×10^{-4}	−1.0 ± 3.4		−0.6
DCl + epinephrine	8.8×10^{-4}	−3.7 ± 1.2	NS	+0.7
DCl + norepinephrine	8.8×10^{-4}	−1.2 ± 2.2	NS	+0.7
DCl + isoproterenol	8.8×10^{-4}	−2.4 ± 3.1	NS	+2.0
Propranolol (prop)	8.8×10^{-4}	+1.8 ± 1.2		+0.1
Prop + epinephrine	8.8×10^{-4}	−1.1 ± 1.1	NS	0.0
Prop + norepinephrine	8.8×10^{-4}	−0.2 ± 0.7	NS	0.0
Prop + isoproterenol	8.8×10^{-4}	+0.5 ± 1.7	NS	−0.2

[a] Each value represents the mean (±SE) of five experiments. After 123 min at 37 C; expressed in microequivalents per gram of tissue.

[b] Values preceded by a + or − sign represent net increase or decrease with respect to control at 0. Absolute values of control tissues in the absence of any exogenous catecholamines averaged 16 μeq of FFA per g.

[c] Significance of increase or decrease compared with control. NS = not significant.

TABLE 3.

Effect of α-adrenergic blocking agents on catecholamine-induced lipolysis

Agent	Molar concn	Net increase in FFA[a]		
		In medium[b]	P[c]	In tissue
None	0	0		0
Epinephrine	1.0×10^{-5}	+13.2 + 2.8	<0.01	+3.1
Norepinephrine	1.1×10^{-5}	+42.0 ± 5.9	<0.01	+11.9
Isoproterenol	1.8×10^{-6}	+32.0 ± 6.0	<0.01	+2.0
Dibenamine (Dba)	2.0×10^{-3}	−3.2 ± 2.3	NS	−0.9
Dba + epinephrine	2.0×10^{-3}	+4.4 ± 1.3	<0.05	+0.5
Dba + norepinephrine	2.0×10^{-3}	+16.4 ± 4.5	<0.05	+4.0
Dba + isoproterenol	2.0×10^{-3}	+12.1 ± 4.3	<0.05	0.0

[a] Each value represents the mean (±SE) of five experiments. After 120 min at 37 C; expressed in microequivalents per gram of tissue.

[b] Values preceded by a + or − sign represent net increase or decrease with respect to control of 0. Control tissues in the absence of exogenous catecholamines averaged 16 μeq of FFA per g.

[c] Significance of increase or decrease compared with control. NS = not significant.

TABLE 4.

Residual epinephrine activity after prolonged incubation

Medium[a]	Epinephrine concn in medium at zero-time	Residual epinephrine activity after 6 hr of incubation (37 C under O_2 phase)	
		In vivo blood glucose levels (mg/100 ml)	In vitro net increase in FFA[c]
SS	0	115 ± 6.0	0.0
SS	1.8×10^{-4} M	110 ± 9.8	−0.7 ± 0.8
SS + 5% albumin	0	108 ± 5.5	0.0
SS + 5% albumin	1.8×10^{-4} M	156 ± 5.4	+14.7 ± 5.1

[a] SS = salt solution, modified Ringers-bicarbonate solution without albumin.

[b] Groups of five mice were injected with 0.2-ml samples of medium from each incubation flask. Blood glucose levels were assayed 30 min postinjection.

[c] After the media had incubated for 6 hr, adipose tissue was added and incubated for an additional 2 hr. Prior to the addition of adipose tissue, 5% albumin was added to those previously albumin-free salt solutions. Expressed in milliequivalents per gram of tissue.

adipose tissue was added to all flasks and fraction V was added to those media that were previously deficient. All flasks were incubated for 2 additional hr and were then assayed for FFA content. The results are shown in Table 4. In the absence of fraction V, epinephrine activity was lost both in terms of FFA release and in the failure to induce hyperglycemia. However, the presence of fraction V appeared to "stabilize" epinephrine so that both responses were retained over the experimental time period.

In addition, experiments with ^{14}C-epinephrine were conducted. The isotopically labeled material was mixed with unlabeled epinephrine so that the final concentration in the incubation medium was 1.8×10^{-5} M. The epinephrine mixtures were incubated in media with and without fraction V. At intervals of 0, 30, 60, and 90 min, samples of equal volume (0.05 ml) were removed for chromatographic analysis as described in Materials and Methods. Figure 3A shows the isotope distribution of samples from the fraction V-free media. The peak representing epinephrine (Ra 1) disappeared after 15 min of incubation, and the majority of radioactivity was localized in two peaks which migrated less rapidly (Ra 0.15 to 0.45) than epinephrine. The presence of fraction V (Fig. 3B), however, appeared to stabilize epinephrine, as it was not appreciably altered after an incubation period of 90 min. Furthermore, incubation for 6 hr in the presence of albumin did not abolish the epinephrine peak (Fig. 3C).

Influence of pertussis vaccine and extract on the

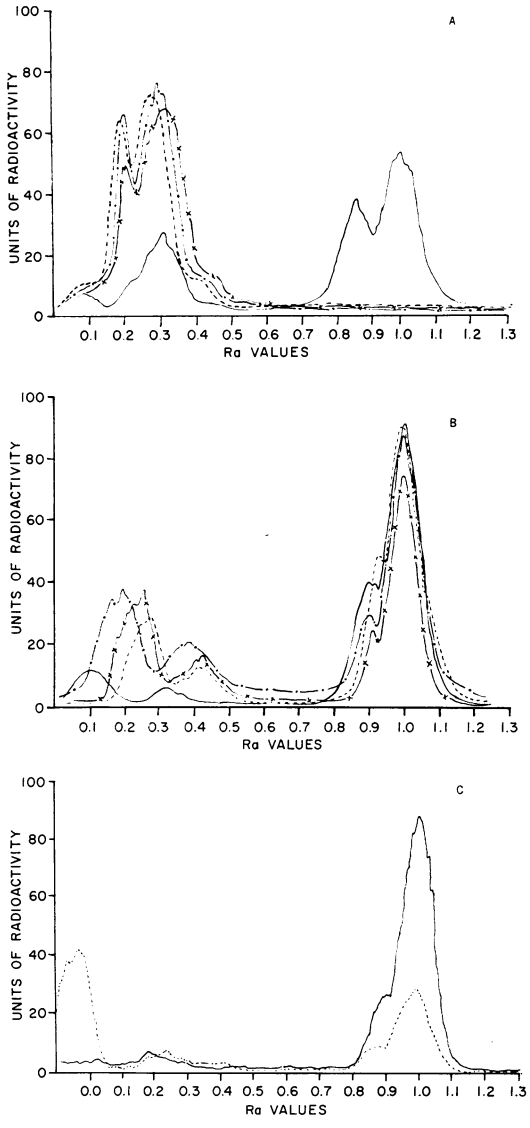

FIG. 3. *(A and B) Distribution of radioactivity during a 90-min period of incubation (37 C under O_2 phase) of ^{14}C-epinephrine in a modified Ringer bicarbonate solution containing (A) no albumin and (B) 5% albumin. Samples taken at time zero, solid line; 30 min, dashed line; 60 min, ——•——; and 90 min, —— × ——. (C) Distribution of radioactivity after incubating (37 C under O_2 phase) ^{14}C-epinephrine for 6 hr in a modified Ringer-bicarbonate solution containing 5% albumin fraction V. Samples taken at time zero, solid line; and 6 hr, dashed line.*

epinephrine-induced FFA responses of adipose tissue. These experiments had two purposes: (i) to test the persistence of adrenergic blockade in adipose tissue removed from vaccinated animals, and (ii) to compare the effect of pertussis vaccine with a soluble extract derived from the organism. Table 5 shows that the FFA response obtained with tissues removed from pertussis-vaccinated animals was essentially the same as that seen with tissues from normal animals.

When normal mouse adipose tissue was preincubated for 15 min in the presence of pertussis vaccine (prior to the addition of epinephrine), no evidence of suppression of the lipolytic response was observed. This was in contrast to the marked antilipolytic effect observed when the tissues were preincubated with the extract.

DISCUSSION

The results in the first part of the present paper confirm some observations noted in a previous report (8). Of the catecholamines employed, epinephrine alone produced hyperglycemia in the normal mouse. The present data may explain the failure of norepinephrine and isoproterenol to elicit a hyperglycemic response in normal members of this species. It has been suggested (30) that elevation of blood glucose is a consequence of two sympathetic phenomena: (i) hepatic glycogenolysis, mediated by α-receptors, contributing glucose, and (ii) glycogenolysis in skeletal muscle, mediated by β-receptors, leading to lactic acid production. As a consequence, the latter could increase the glucose level by making available lactic acid (and FFA) for glycogen synthesis, and by decreasing the peripheral uptake of glucose through inhibition of glucokinase enzyme (7). The synergistic effects of α- and β-receptors have also been shown by Lei and McCutcheon (14). The failure of norepinephrine to stimulate hyperglycemia might then be due to suppressed action on receptors at the skeletal muscle level with isoproterenol ineffective at hepatic sites.

Selective blockade of the adrenergic receptors in vivo (Table 1) served two purposes. Firstly, β-receptor interference suppressed the FFA and lactic acid response to the normally active amines which would implicate skeletal muscle and adipose tissue sites. Blockade of the α-receptors prevented hepatic glycogenolytic activity. Thus, the types of adrenergic receptors involved in these metabolic events in the mouse appear to be rather discrete. Secondly, the data re-emphasize the similarities between β adrenergically blocked and pertussis-sensitized animals. The catecholamines, epinephrine and norepinephrine, induce hepatic glycogenolysis in normal as well as pertussis-sensitized and β-blocked mice, but not in α-blocked animals. Epinephrine and isoproterenol, which elevate serum FFA in normal and α-blocked mice, fail to do so in β-blocked and pertussis-sensitized groups. Similarly, pertussis-

TABLE 5.

Influence of pertussis vaccine and extract on the epinephrine-induced FFA response of adipose tissue

Source of tissue	Additions to medium	FFA in medium[a]
Vaccinated mice	Epinephrine (8×10^{-5} M)	25.2 ± 2.1
Normal mice	Epinephrine (8×10^{-5} M)	27.3 ± 3.0
Normal mice	Pertussis vaccine (0.05 ml) + epinephrine	26.4 ± 3.6
Normal mice	Pertussis extract (0.01 mg) + epinephrine	1.9 ± 0.3

[a] Mean ± SE of five separate determinations, expressed in microequivalents per gram of tissue.

sensitized and β-blocked mice are unresponsive to those catecholamines whose administration is normally followed by a hyperlactic acidemia.

Up to this point, the data suggesting the presence of β-adrenergic blockade in pertussis-sensitized mice was derived from in vivo experiments. These data were not adequate, however, to resolve the question of whether malfunction was due to a bacterial component or to a substance elaborated by the host (9). To gain further insight into both aspects, in vitro experiments were undertaken. Adipose tissue was selected for several reasons: (i) it can be obtained with minimal effort; (ii) incubation procedures are well established as are methods to test response (27, 31); and (iii) the in vivo data indicated the adrenergic unresponsiveness of this tissue in sensitized animals. The adipokinetic activity of various hormones as well as the influence of blocking agents on adrenergic response (17, 23) has been reported for adipose tissue derived from a variety of species (27, 28, 31). Divergent interpretations of these data relative to the basic mechanism mediating lipolysis, as well as observed differences in responsiveness between species, complicate the assignment of a uniform receptor type common to all species.

With these considerations in mind, a comparison (Fig. 1) of the relative potencies of epinephrine, norepinephrine, and isoproterenol (isoproterenol > norepinephrine > epinephrine), coupled with efficiency of blockade by propranolol (Fig. 2), suggests at least the participation of a β-type receptor in the catecholamine-induced release of FFA from mouse adipose tissue. It was of concern whether the relative potencies of the catecholamines might not represent differences in the rates of catabolism of substances during the incubation period of 2 hr. With epinephrine, this did not appear to be the case since the presence of bovine serum albumin (fraction V) appeared to stabilize the hormone for at least 6 hr, whereas activity was lost rapidly in the absence of the fraction. The ability of whole blood (5) and more specifically of Cohn-fraction V derived from human plasma (2) to bind and stabilize catecholamines has been demonstrated by a variety of procedures. Mirkin et al. (18) found that tritium-labeled norepinephrine was bound by a human plasma protein electrophoretically resembling a β-globulin or haptoglobin. To our knowledge, however, the present data are unique in demonstrating the epinephrine-stabilizing effect in vitro of bovine serum fraction V in terms of both chromatographic distribution of radioactive fractions and retention of physiological activity for the period of time involved in the incubation procedure. We are currently examining the binding of various catecholamines with serum fractions of other animal species.

The suppression of catecholamine-induced FFA released by pertussis extract (Table 5) provides the first in vitro evidence of a bacterial substance capable of blocking adrenergic receptors. It is tempting to relate this effect to specific action on the β-receptor. However, owing to the dose dependency of specific receptor response, as noted previously, it is not possible at the present time to make such an assignment. On the other hand, in the absence of any previous or present evidence implicating α-receptors, the suppressive effect of the extract strongly suggests a β-receptor blockade.

It has been found by others that removal of adipose tissue from adrenergically blocked animals also removed the blocking effect (16). These authors also demonstrated that tissue taken from an in vitro medium containing a blocking compound and placed in a medium free from the latter regained normal catecholamine responsiveness. Therefore, failure to demonstrate adrenergic blockade in adipose tissue removed from pertussis-sensitized mice (Table 5) does not militate against the present concept. The inability of pertussis vaccine to interfere with FFA release is believed to be related to the nature of the particulate suspension.

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