Rapid Publications

Anti-Sympathetic Nervous System Autoantibodies

Diminished Catecholamines With Orthostasis

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The etiology of autonomic neuropathy in insulin-dependent diabetes mellitus (IDDM) is unknown. Previous studies have noted the presence of anti-adrenal medullary antibodies in IDDM. Recently, we have also demonstrated the presence of anti-sympathetic ganglia antibodies in IDDM. We initiated a study to evaluate whether subjects with complement-fixing anti-adrenal medullary (CF-ADM) and anti-sympathetic ganglia (CF-SG) antibodies have a decreased catecholamine response to change in posture. Seven IDDM subjects aged 19–41 yr with duration of disease 5–21 yr at the time of the posture study were evaluated. Serums collected longitudinally were evaluated for the presence of CF-ADM and CF-SG antibodies. Three IDDM subjects were CF-ADM$^-$ and CF-SG$^-$ at all testing intervals (Ab$^-$ group). Four IDDM subjects were CF-ADM$^+$ and/or CF-SG$^+$ on at least one testing date (Ab$^+$ group). Baseline mean norepinephrine and epinephrine levels were not significantly different in Ab$^+$ and Ab$^-$ subjects. Norepinephrine levels 5 min after standing were mean ± SD 227 ± 16 and 419 ± 48 pg/ml for Ab$^+$ and Ab$^-$ subjects, respectively ($P < .03$). The means of the 5-min minus basal norepinephrine levels were 88 ± 42 (Ab$^+$) and 207 ± 26 (Ab$^-$) pg/ml ($P < .03$). Mean epinephrine levels after 5 min of standing were 35 ± 16 (Ab$^+$) and 101 ± 44 (Ab$^-$) pg/ml ($P < .03$). The means of the 5-min minus basal epinephrine levels were 1 ± 5 (Ab$^+$) and 43 ± 38 (Ab$^-$) pg/ml ($P < .03$). Mean change in systolic blood pressure on standing was not different in the two groups. This suggests that CF-ADM and CF-SG are associated with a decreased catecholamine response to change in posture.
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Autonomic nervous system dysfunction is a well-known complication of insulin-dependent diabetes mellitus (IDDM) occurring in 20–40% of consecutively studied subjects (1). Subclinical autonomic dysfunction may be present for years before overt signs and symptoms develop. The cause of autonomic neuropathy is unknown. Duchen et al. (2) noted the presence of a lymphocytic infiltrate in the sympathetic ganglia of five IDDM subjects with autonomic neuropathy. Decreased basal and/or standing catecholamines have been described in subjects with autonomic neuropathy (3–5).

Previous studies have noted the presence of anti-adrenal medullary antibodies in IDDM (6–8) and subjects at high risk of developing IDDM (8). We have also noted the presence of anti-sympathetic ganglia antibodies in IDDM subjects (9). We initiated this study to evaluate whether subjects with complement-fixing anti-adrenal medullary (CF-ADM) and anti-sympathetic ganglia (CF-SG) antibodies have diminished catecholamine response to change in posture.

RESEARCH DESIGN AND METHODS

All subjects gave informed consent before participating in the study. A computer data base was constructed from ~3000 randomly stored serum samples from the diabetes practice of S.J.B. Subjects' serums were selected for evaluation of CF-SG and CF-ADM if multiple serum samples were available over a >2-yr period. Subjects were selected from this group for evaluation of autonomic function if they were >18 yr of age and had duration of diabetes of >5 yr.

Seven IDDM subjects aged 19–41 yr (mean ± SD 25.9 ± 7.7 yr) had diabetes for 5–21 yr (mean ± SD 11.0 ± 5.5 yr) at the time of the posture study. None of the subjects were selected on the basis of presence or absence of known clinical autonomic neuropathy.

Three to five serum samples were available for analysis from each of the seven subjects over a 2- to 8-yr period. Serum was stored at −20°C until the time of assay.

Assay for CF-ADM and CF-SG antibodies. An indirect immunofluorescence complement-fixation technique was used as previously described (8,9). Human adrenal gland was used as substrate for the anti-adrenal medullary antibody assay, and rabbit sympathetic ganglia was used as substrate for the anti-sympathetic ganglia assay. Fresh human serum was used as a complement source. Fluorescein isothiocyanate goat anti-human C3c (Calbiochem-Behring, LaJolla, CA) was used as the detecting agent. Sections were tested in a blinded fashion and scored as previously described (8,9).

Assessment of clinical neuropathy. Screening of the peripheral nervous system included evaluation of reflexes, pinprick, and/or vibration sensation. Autonomic testing included heart-rate variation with respiration and Valsalva maneuver and 30:15 ratios after standing according to the method of Ewing et al. (10). The difference between the maximum and minimum heart rate with respiration was considered normal if ≥15 beats/min, borderline if 11–14 beats/min, and abnormal if ≤10 beats/min (10). The Valsalva ratio (ratio of maximum heart rate during expiratory phase to minimum heart rate during relaxation phase) was normal if ≥1.21, borderline if 1.11–1.20, and abnormal if ≤1.10 (10). The ratio of the longest R-R interval at the 30th beat to the shortest R-R interval at the 15th beat after standing was normal if ≥1.04, borderline if 1.01–1.03, and abnormal if ≤1.00 (10). Symptoms of autonomic and peripheral neuropathy were assessed by following the questionnaire of Dyck (11).

Posture study. An intravenous catheter was inserted at −45 min. The subject remained supine for 45 min before standing. Plasma epinephrine and norepinephrine were determined at time 0 (supine) and after 5 min of upright posture.

Catecholamine assay. Measurement of plasma epinephrine and norepinephrine was determined with a simultaneous single-isotope radioenzymatic assay as previously described (12). Catechol *O*-methyltransferase was used as a catalyst in the transfer of a radioactive methyl group from *S*-adenosyl-L-methionine to the endogenous plasma catecholamine molecule. Separation of [^{3}H]normetanephrine and [^{3}H]metanephrine derivatives was achieved by rapid thin-layer chromatography.

Statistics. Comparisons between groups were evaluated with Fisher's exact test.

RESULTS

Three IDDM subjects aged 21–25 yr (22.3 ± 2.3 yr) with 5–21 yr duration of diabetes (13.0 ± 8 yr) were CF-ADM$^-$ and CF-SG$^-$ at all testing intervals (Ab$^-$ group). Four IDDM subjects aged 19–41 yr (28.5 ± 9.7 yr) with 6.5–14 yr duration of diabetes (9.6 ± 3.3 yr) were CF-ADM$^+$ and/or CF-SG$^+$ on at least one testing date (Ab$^+$ group) (Table 1). Results of plasma catecholamine levels in Ab$^+$ versus Ab$^-$ subjects are shown in Figs. 1 and 2. Mean baseline norepinephrine levels were not significantly different in Ab$^+$ and Ab$^-$ subjects (199 ± 69 vs. 212 ± 92 pg/ml, respectively). Similarly, mean ± SD baseline epinephrine levels of 31 ± 16 pg/ml in Ab$^+$ subjects did not differ significantly from 58 ± 23 pg/ml in Ab$^-$ subjects. Mean norepinephrine levels after 5 min of standing were 227 ± 16 pg/ml (Ab$^+$) and 419 ± 48 pg/ml (Ab$^-$) ($P < .03$). The means of the 5-min minus basal norepinephrine levels were 88 ± 42 pg/ml (Ab$^+$) and 207 ± 26 pg/ml (Ab$^-$) ($P < .03$). Mean epinephrine levels after 5 min of standing were 35 ± 16 pg/ml (Ab$^+$) and 101 ± 44 pg/ml (Ab$^-$) ($P < .03$). The means of the 5-min minus basal epinephrine levels were 1 ± 5 pg/ml (Ab$^+$) and 43 ± 28 pg/ml (Ab$^-$), respectively ($P < .03$).

TABLE 1
Subject characteristics

Subjects	CF-ADM	CF-SG	5-min norepinephrine (pg/ml)	5-min epinephrine (pg/ml)
1	−	−	427	150
2	−	−	367	66
3	−	−	463	88
4	+	+	167	<10
5	−	+	292	43
6	−	+	278	45
7	+	−	171	30

CF-ADM, complement-fixing anti–adrenal medullary antibodies; CF-SG, anti–sympathetic ganglia antibodies. −, Absent; +, present.

All subjects denied symptoms of peripheral and autonomic neuropathy. Subject 7 had hypoglycemia unawareness (Table 1). This subject also had borderline abnormal heart-rate variation with respiration (maximum-minimum heart rate 11.3 beats/min) and CF-ADM. Cardiovascular autonomic test results, except tests of orthostasis and catecholamine levels, were unavailable for subject 1. The rest of the subjects had normal heart-rate variation with respiration and Valsalva maneuver and normal 30:15 ratios with upright posture. All subjects had normal reflexes, pinprick, and/or vibration sensation. None of the subjects had orthostatic hypotension during the study. The mean change in systolic blood pressure on standing was +14 mmHg in both groups.

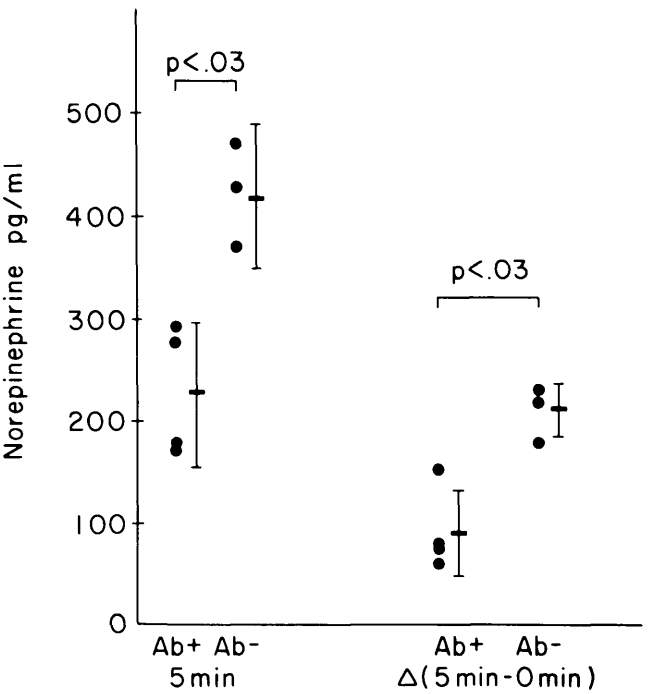

FIG. 1. Norepinephrine levels after 5 min of standing and at 5 min minus basal (Δ) in antibody (Ab)$^+$ and Ab$^-$ subjects.

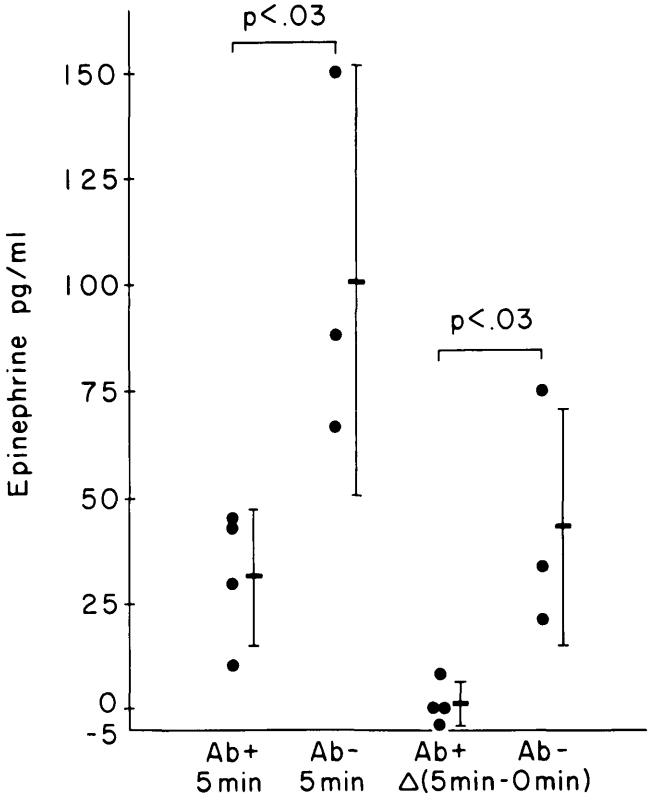

FIG. 2. Epinephrine levels in antibody (Ab)$^+$ and Ab$^-$ subjects after 5 min of standing, and epinephrine levels at 5-min minus basal (△) in Ab$^+$ and Ab$^-$ subjects.

Serum from a 19-yr-old subject with 6 yr of IDDM was collected 1, 5, and 6 yr after onset of IDDM. CF-ADM and/or CF-SG were present in serums on three of three occasions. His catecholamine levels were the lowest of all subjects studied. Five-minute norepinephrine and epinephrine levels were 167 and <10 pg/ml (below the sensitivity of the assay). Five-minute minus basal norepinephrine and epinephrine levels were 73 and 0 pg/ml, respectively.

DISCUSSION

We demonstrated diminished norepinephrine and epinephrine levels after assumption of upright posture in IDDM subjects with CF-ADM and CF-SG compared with IDDM subjects without these antibodies. Serial serum samples were collected over 2–8 yr in the subjects. Although basal catecholamines were not significantly decreased in the two groups, both norepinephrine and epinephrine levels after 5 min of standing were significantly decreased in the Ab$^+$ group. Blunted norepinephrine responses to standing with normal supine norepinephrine levels have been noted in subjects with primary autonomic neuropathy (4). Other studies have demonstrated diminished basal and standing catecholamines in subjects with diabetes of long duration and autonomic neuropathy (3,5).

We have recently demonstrated an increased prevalence of CF-SG in subjects whose blood pressure response to standing was in the lower portion of the normal response range (mean to −2SD; 9), suggesting a possible role for the immune system in the development of autonomic neuropathy. Recently, Scherbaum et al. (13) were unable to find an association between CF-ADM and supine catecholamine levels in new-onset IDDM subjects. We chose to study subjects with 5–21 yr duration of IDDM, because the incidence of clinical and subclinical autonomic neuropathy increases in subjects with longer duration of IDDM.

Two of four subjects in the Ab$^+$ group had only CF-SG, 1 subject had only CF-ADM, and 1 subject had CF-ADM and CF-SG. Postganglionic sympathetic neurons secrete norepinephrine, and the adrenal medulla secretes ~80% epinephrine and 20% norepinephrine; therefore, it might be expected that subjects with CF-SG would have a defect in norepinephrine, whereas subjects with CF-ADM would have a defect primarily in epinephrine response to upright posture. Possible explanations for the abnormalities in both epinephrine and norepinephrine response in patients with either CF-ADM or CF-SG include the fact that the adrenal medulla is innervated by preganglionic sympathetic nerve fibers (14). A defect in the preganglionic sympathetic nervous innervation of the adrenal medulla could result in a decrease in epinephrine secretion. Although controversial, it is also possible that epinephrine release from the adrenal medulla may enhance the release of norepinephrine from sympathetic nerve terminals by activating prejunctional β-adrenoreceptors. This might account for the diminished norepinephrine secretion in our patient with only CF-ADM (15–17).

It is also possible that although our antibody assays are highly specific, they may not be sensitive enough to pick up all patients who have low titers of these antibodies (8,9). This is similar to the description in the literature that ~60–80% of new-onset IDDM subjects have detectable islet cell antibodies (18–20).

Despite the fact that there was no difference in blood pressure response to standing, we were able to demonstrate a difference in catecholamine levels with standing in the two groups. Subject 14 had a systolic blood pressure response to standing that fell between mean to −2SD below normal and was CF-SG$^+$ and CF-ADM$^+$ on multiple occasions (Table 1). His catecholamine levels were the lowest of all subjects tested. Subject 7 had several episodes of neuroglycopenia without warning symptoms, borderline abnormal heart-rate response to respiration, and CF-ADM (Table 1). Continued prospective follow-up is necessary to determine whether subjects with CF-SG and CF-ADM will ultimately develop autonomic dysfunction, orthostatic hypotension, and severe catecholamine deficiency.

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