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DETERMINATION OF INTRAOPERATIVE PLASMA CATECHOLAMINE CONCENTRATIONS USING LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

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SUMMARY

Liquid chromatography with electrochemical detection was used for the determination of norepinephrine and epinephrine in human plasma samples obtained prior to, after, and six times during the course of spinal fusion surgery for the correction of scoliosis. The catecholamines were extracted from plasma by alumina adsorption and chromatographed isocratically using a reversed-phase, ion-pairing system. Data obtained are compared to those obtained intraoperatively by other authors using a radioenzymatic method, and the mechanism of sympathetic activation during surgery is discussed. Preliminary data using 3-μm particle size columns and dual-parallel electrochemical detection are presented.

INTRODUCTION

Surgical stress in man is characterized by definite neuroendocrine responses, including release of ACTH, growth hormone, prolactin and β-endorphin [1—5]. The sympathetic nervous system (SNS) is also considered to be an important mediator of stress responses [6, 7]. However, earlier studies, using estimates of urinary catecholamine excretion or fluorometric analysis of plasma catecholamines during surgery have yielded conflicting results [8—10]. More recently, the use of sensitive radioenzymatic methods for the measurement of plasma catecholamines have demonstrated consistent

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increases in SNS activity during surgical stress following anesthesia-induced depressions in activity [11]. Whereas the sensitivity of radioenzymatic methods is unsurpassed, these methods are expensive and labor-intensive and therefore do not lend themselves to routine clinical analyses. In order to circumvent this problem, we have developed a selective and sensitive method utilizing liquid chromatography (LC) with electrochemical detection (ED), to measure the concentration of catecholamines in plasma. We have previously applied this method to the determination of norepinephrine in brain perfusates [12]. LC methods have been successfully applied to the determination of catecholamines in plasma [13--16]. LC—ED methods for the determination of plasma catecholamines have been shown to correlate very well with radioenzymatic methods [16] and are superior to the latter in the presence of elevated plasma dopamine concentrations [17].

The present study was designed to evaluate the utility of LC—ED methodology for the determination of plasma catecholamines during the course of surgery (posterior spinal fusion for the correction of scoliosis). Since surgical procedures from the induction of anesthesia to closure have been shown to be accompanied by both decrements and elevations of SNS activity [11], it was felt that the determination of plasma catecholamines under these conditions would provide a good test of the feasibility of LC—ED for both routine and non-routine clinical analyses.

EXPERIMENTAL

Subjects

Nine patients (eight females, one male) admitted to the hospital for elective posterior spinal fusions were included in this study. Their ages ranged from 12 to 21 years (mean: 17 years). All patients were in satisfactory general health, as judged by clinical testing and physical examination (physical status: ASA

TABLE I

PATIENTS CHARACTERISTICS, BLOOD LOSSES AND TRANSFUSIONS, FLUIDS GIVEN, BLOOD GASES

Patient	Age (years + months)	Sex	Procedure	Estimated blood loss (ml)	Blood transfusions (ml)	Fluids given (ml)	Blood gases at post-graft (mmHg)	
							pO_2	pCO_2
1	18 + 8	M	Posterior spinal fusion	1222	450	800	172.0	29.0
2	12 + 1	F	Posterior spinal fusion with Harrington rod	826	810	800	172.3	28.9
3	19 + 2	F	Posterior spinal fusion with Harrington rod	300	450	950	155.3	31.8
4	21 + 4	F	Posterior spinal fusion	1000	810	1100	158.8	27.2
5	18 + 8	F	Posterior spinal fusion with two Harrington rods	776	900	1150	124.8	27.7
6	13 + 11	F	Posterior spinal fusion with Harrington rod	450	405	900	182.0	24.8
7	18 + 11	F	Posterolateral spinal fusion	1060	900	1050	165.0	26.0
8	15 + 7	F	Posterior spinal fusion	199	0	650	192.0	24.0
9	13 + 2	F	Posterior spinal fusion	600	450	700	171.0	32.0

Class I). None of the patients were taking monoamine oxidase inhibitors or tricyclic antidepressants and none had a history of narcotic drug abuse. Informed consent was obtained from each patient prior to the study. Relevant patient information is shown in Table I.

Anesthetic management

Following an after-midnight fast, the patients were premedicated for anesthesia 1 h prior to surgery with sodium pentobarbital (2 mg/kg), morphine sulfate (0.12 mg/kg), and scopolamine (0.2—0.3 mg). An intravenous catheter (angiocath size 10) was established with 5% dextrose in 0.2% saline. Droperidol (0.05—0.10 mg/kg; total dose: 2.5—5 mg), fentanyl (2.0—5.0 μg/kg), sodium thiopental (4—6 mg/kg), succinylcholine (100 mg), and *d*-tubocurarine (0.4—0.6 mg/kg), were subsequently administered through this catheter. Blood pressure was monitored via a pressure transducer connected to a catheter (angiocath, size 20) inserted into the radial artery. Following tracheal intubation, ventilation was controlled with an Ohio anesthesia ventilator (Ohio Medical Products, Madison, WI, U.S.A.) at a total volume (10—12 ml/kg) and rate sufficient to maintain P_{CO_2} at a level of 25—30 mmHg. Anesthesia was maintained by a combination of nitrous oxide—oxygen (66:33) and halothane (0.5%). Surgery was accomplished with the patient placed in the prone position on a supporting frame. Following closure, neuromuscular blockade was antagonized with neostigmine (2.5 mg) and atropine (1.0 mg). Extubation was accomplished once ventilation was satisfactory.

Mean arterial pressure (calculated as diastolic pressure plus 1/3 [systolic—diastolic]), heart rate, and body temperature (measured at the tympanic membrane with a LaBarge Mon-A-Therm electrode (LaBarge, St. Louis, MO, U.S.A.) were recorded each time a blood sample was obtained. Arterial P_{O_2} and P_{CO_2} were measured once during the sampling period, following the bone graft procedure. Blood loss was estimated on a continuing basis by weighing the gauze sponges (after subtracting tare weight) removed from the surgical field and by measuring the volume of blood suctioned from the field. Blood volume was replaced as necessary with saline—dextrose and/or whole blood.

Sampling

The first sample of blood was drawn in the pre-surgical holding area prior to induction of anesthesia, using the arterial line. The patient had been in a supine position for approximately 30 min at which time 3—5 ml of blood were drawn and discarded. The pre-induction blood sample (9 ml) was then withdrawn into two heparinized tubes, each containing 75 mg Na_2EDTA. The sample was placed on ice and brought to the laboratory within 10 min. The sample was then centrifuged at 2000 *g* for 20 min at 4°C. The resulting plasma supernatant was stored at −90°C until analysis. All samples were analyzed within two weeks of collection. In preliminary studies, we had established that within this time period, no degradative loss of catecholamines occurs at −90°C. Subsequent samples were handled identically and were withdrawn just after induction of anesthesia when blood pressure and heart rate were stable, 10 min after the initial incision, after stripping the musculature overlying the vertebral colum, after obtaining a bone graft, after decortication of the spinous processes, after closure and finally in the recovery room.

Reagents

Norepinephrine (NE), epinephrine (E) and dopamine (DA) (Sigma, St. Louis, MO, U.S.A.), 3,4-dihydroxybenzylamine (DHBA) (Aldrich, St. Regis, WI, U.S.A.), and sodium octyl sulfate (Eastman, Rochester, NY, U.S.A.) were used. Acid-washed activated alumina was purchased from Bioanalytical Systems (West Lafayette, IN, U.S.A.) but was customarily re-activated according to the method of Anton and Sayre [18]. All other chemicals were of reagent grade and solutions were prepared from HPLC-grade water obtained from Fisher (Fairlawn, NJ, U.S.A.). All solutions were filtered through a 0.22-μm membrane filter (Millipore, Bedford, MA, U.S.A.) before use.

Stock solutions of catecholamines and DHBA, 1 mg/ml in 0.1 *M* perchloric acid containing 1.3 m*M* EDTA and 7.9 m*M* sodium metabisulfite, were prepared bimonthly and stored at 4°C. Working standards were prepared daily by making appropriate dilutions of the stock solutions in perchloric acid. Solutions of 1.5 *M* Tris (pH 8.6) and 0.1 *M* phosphate buffer (pH 7.4), both containing 52 m*M* EDTA, were prepared monthly.

Extraction of catecholamines

Prior to the (LC—ED) analysis, plasma aliquots (0.4—3 ml), were extracted using alumina. Plasma, or synthetic standards prepared in 0.1 *M* phosphate buffer (pH 7.4) and internal standard, dihydroxybenzylamine (DHBA, 4 ng per 20 μl of 0.1 *M* perchloric acid solution) were added to 15-ml conical polypropylene tubes containing 20 mg acid-washed alumina (baked at 180°C for 3 h prior to use), and extracted as in previous work [12, 18]. After washing the alumina with water twice, an alumina—water slurry was transferred to a centrifugal microfilter (Bioanalytical Systems) containing a 0.2-μm nitrocellulose or Nylon-66 membrane filter. The alumina was dried by centrifugation at 2500 *g* for 1 min.

The catecholamines were then eluted by adding 100 μl of the 0.1 *M* perchloric acid solution and vortexing for 1 min. This slurry was then centrifuged at 2500 *g* for 1 min yielding the final solution.

Chromatography

A Hewlett-Packard 1084B system was used (Hewlett-Packard, King of Prussia, PA, U.S.A.); it included two reciprocating diaphragm pumps, a continuously adjustable injection system, a Supelco LC-18 column (5 μm, C_{18}-bonded silica, 250 × 4.6 mm I.D.) and precolumn (50 × 4.6 mm I.D.) (Supelco, Bellefonte, PA, U.S.A.) maintained at a constant temperature of 30°C, or 45°C (for NE, E and DA, respectively) and a Bioanalytical Systems TL5 electrochemical cell with glassy carbon electrode and LC4 amperometric controller. Chromatograms were recorded on both the Hewlett-Packard graphic integrator and a Houston Omniscribe potentiometric recorder (Houston Instruments, Austin, TX, U.S.A.).

The mobile phase was compsed of 14.5 m*M* citric acid, 71.0 m*M* dibasic sodium phosphate, 1.3 m*M* EDTA and $1.7 \cdot 10^{-4}$ *M* sodium octyl sulfate. The pH was adjusted to 6.0 with 2 *M* sodium hydroxide. This pH value results in a greater retention of bases by reversed-phase columns and an improved detector response [19] as compared to more acidic mobile phases. The flow-rate was

1.0 ml/min. The mobile phase was filtered through a 0.22-μm filter and degassed in vacuo and by helium purging. The working electrode potential was +0.9 V vs. a Ag/AgCl reference electrode. An 85-μl aliquot of the final 100-μl alumina extract was injected onto the column.

Preliminary analyses were also performed on 3-μm particle size reversed-phase columns. For these analyses, a BAS LC 304 system was used with a Supelco LC-18 column (3 μm, C_{18}-bonded silica, 75 × 4.6 mm I.D.) and guard column (Supelguard, 5 μm, 20 × 4.6 mm I.D.) joined with a direct connect coupler (Alltech Assoc., Waukegan, IL, U.S.A.). Because extra column effects can be significant with these columns, several modifications in system plumbing had to be made. A 3-cm length of 0.25-mm (0.01 in.) I.D. tubing was used to connect the guard—analytical column assembly to the injection valve. A 5-cm length of 0.13-cm (0.005 in.) I.D. tubing connected the column outlet to the detector inlet. The latter end was fitted with a plastic compression fitting (Upchurch Scientific, Oak Harbor, WA, U.S.A.). The overall distance between injector outlet and detector inlet was approximately 20 cm. Finally, a low dead volume, stainless-steel detector top was used (Bioanalytical Systems). Other chromatographic components were the same.

The above mobile phase was slightly modified for the short columns by the addition of 0.13 m*M* diethylamine (Sigma; 1.28 ml of solution as supplied per l of mobile phase). The diethylamine was added to the mobile phase system when used with short columns in order to mask unbonded silanol groups on the column and thus reduce peak tailing. Since diethylamine is a strong base, it was necessary to add phosphoric acid to the mobile phase to regain the intended pH value of 6.0. The concentrations of citric acid and sodium phosphate were halved. This mobile phase was delivered at 2.0 ml/min. In these analyses, a dual-electrode electrochemical detector (BAS, MF 1000) was employed in the parallel mode. Applied potentials were +0.70 V and +0.80 V vs. Ag/AgCl, respectively. In addition, the detector outputs were filtered by means of internal three-pole Butterworth active filters at 0.1 Hz and 0.3 Hz, respectively. For analysis on short columns, 20-μl injections were used. A final modification made for chromatographic analysis on short columns was the use of 1 ng DHBA as opposed to 4 ng due to the higher efficiency of these columns.

Quantification of catecholamine concentrations

The concentrations of NE, E and DA in each sample, were calculated by determining their peak height ratios relative to DHBA and comparing these ratios to those obtained with synthetic standards prepared in 0.1 *M* phosphate buffer (pH 7.4). These synthetic standards were prepared in three different concentrations in the range of expected sample values and carried through the same procedure as the samples. In addition, the relative recovery of catecholamines was determined using plasma pools fortified with reference solutions of NE, E and DA. Because dopamine (DA) was detected in only 12 of 72 samples using conventional columns, data on this catecholamine are not included in this report. All plasma catecholamine concentrations are reported as the mean of duplicate determinations. All duplicate measurements were within the limits of variation noted in the Results section. A third determination was employed

for the measurement of DA at 45°C. Data were analyzed using standard statistical methods that included two-tailed *t*-tests and linear regression analysis [20] preceded by preliminary data analysis using univariate analysis of variance of repeated measurements [21]. Catecholamine concentrations are expressed as pg/ml of plasma.

RESULTS

Sample chromatograms

Fig. 1 illustrates chromatograms of a synthetic standard and two 3-ml plasma samples obtained intraoperatively. All samples were spiked with 4 ng of DHBA. Each chromatogram was obtained at 30°C. If the presence of DA peak was noted, the triplicate sample was chromatographed at 45°C in order to obtain a capacity factor for DA of approximately 5 (retention time of 24 min). Only 12 of 72 samples assayed displayed detectable quantities of DA. At 45°C, the NE peak was indistinguishable form the elution front. Fig. 2 is an example of the chromatograms obtained using the short, 3-μm particle size column. Note the expansion of the time scale as compared to Fig. 1, and the low elution volume of each peak. Also note the resolution of all three catecholamines and DHBA within 7 min. It is estimated that the combination of the short column with active filtering of the detector output can result in a ten-fold lowering of the detection limit depending on the compound analyzed.

Precision and relative recovery

Repeated determinations (n = 30) of NE, E and DA in 3 ml of 0.1 *M* phosphate buffer gave the following coefficients of variation (C.V.): NE, C.V. = 3.4% at a concentration of 300 pg/ml; E, C.V. = 4.8% at a concentration of 90 pg/ml; DA, C.V. = 4.6% at a concentration of 80 pg/ml (column temperature

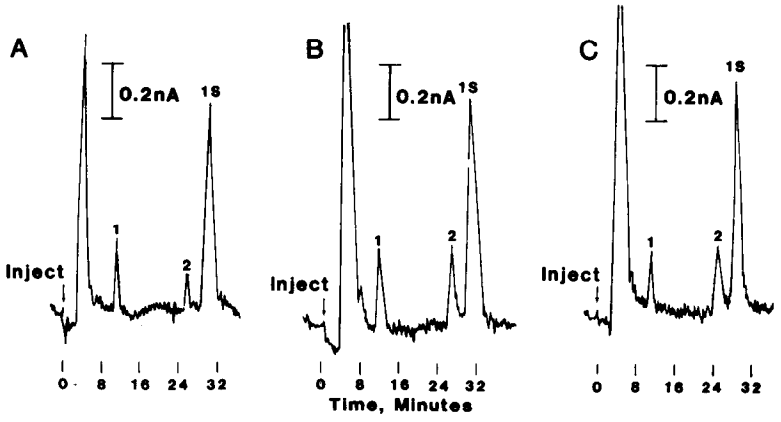

Fig. 1. Sample chromatograms obtained by the present LC—ED method, using a 5-μm particle size column. (A) Synthetic standard composed of 3 ml of 0.1 *M* phosphate buffer with 300 pg/ml NE and E; (B) 3-ml plasma sample taken at post-decortication containing 409 pg/ml NE and 743 pg/ml E; and (C) 3-ml plasma sample taken at closure containing 262 pg/ml NE and 665 pg/ml E. A column temperature of 30°C was used, glassy carbon electrode at +0.90 V vs. Ag/AgCl. Peaks: 1 = NE; 2 = E; and IS = internal standard, DHBA.

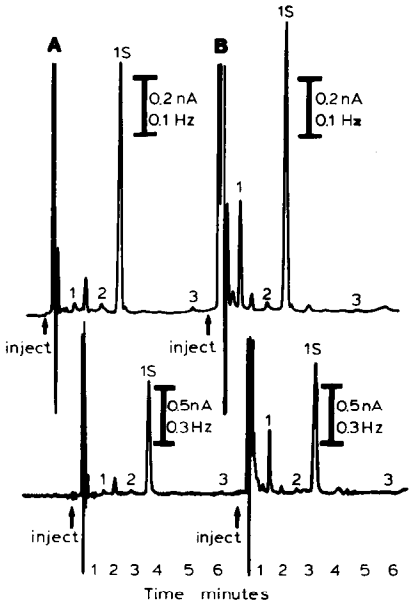

Fig. 2. Sample dual chromatograms obtained by the present LC—ED method, using a 3-μm particle size column, illustrating improved detection limits. (A) Synthetic standard composed of 1 ml of 0.1 *M* phosphate buffer with 50 pg/ml NE, E and DA. With the volume injected and absolute recovery (based on the internal standard), peaks correspond to ca. 7 pg injected. (B) Pre-induction plasma sample from patient No. 4 (see Tables II and III). Chromatogram is a repeat determination using 0.9 ml plasma and contains 561 pg/ml NE and 42 pg/ml E, in close agreement to the values determined on the 5-μm column. The DA peak near the limit of detection corresponds to 25 pg/ml. Column temperature was ambient. Upper traces recorded at +0.70 V, bottom traces at +0.80 V vs. Ag/AgCl. Sensitivities and filter settings noted in vertical calibrations. Increasing the sensitivity in the lower trace would allow accurate quantification of DA at two potentials. Note expansion of time scale vs. Fig. 1. Peaks: 1 = NE; 2 = E; 3 = DA; and IS = internal standard, DHBA.

30°C for NE, E and 45°C for DA). The between-day coefficients of variation were determined from the same standard concentrations and were: NE, C.V. = 7.1%; E, C.V. = 8.8% and DA, C.V. = 10.2%. Preliminary analyses on 3-μm columns employing dual-electrode detection were characterized by lesser variability in the determination of E and DA.

The relative recoveries of catecholamines from outdated blood bank plasma as compared to the 0.1 *M* phosphate buffer, at the concentrations noted above, were: NE = 91.5 ± 2.6%, E = 84.3 ± 4.4% and DA = 79.2 ± 6.1% (n = 20). When the internal standard was included in the calculations, all values approached 95%.

Linear range and limits of detection

These data were determined from 3-ml 0.1 *M* phosphate buffer aliquots fortified with catecholamines. The assay was linear in the range 30 pg/ml–50 ng/ml for NE; 45 pg/ml—50 ng/ml for E and 50 pg/ml–10 ng/ml for DA. The minimum detectable quantity injected, at a signal-to-noise ratio of 2:1, was 38 pg of NE, 55 pg of E and 63 pg of DA. It should be noted that these sensitivities could only be achieved when the noise level averaged 0.01 nA.

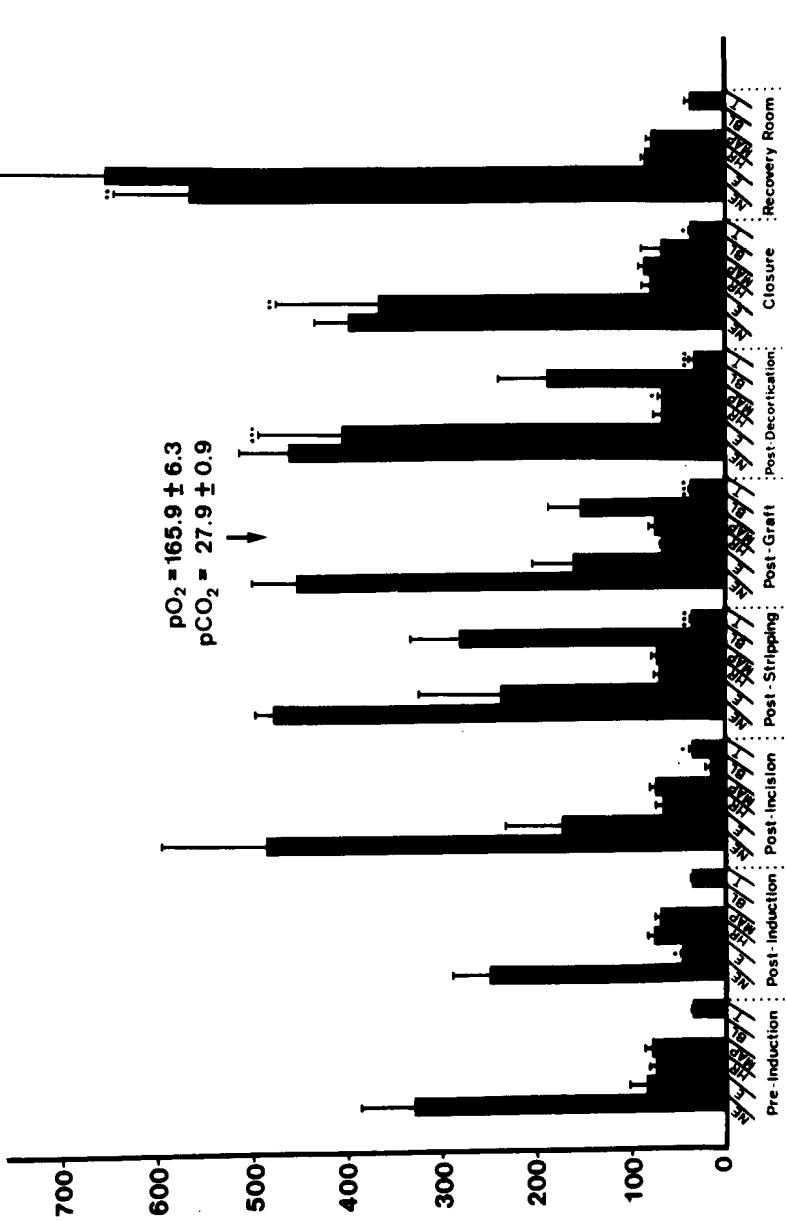

Fig. 3. Mean values + S.E.M. for plasma norepinephrine (NE) and epinephrine (E) concentrations (pg/ml), heart rate (HR) (beats/min), mean arterial pressure (MAP) (mmHg), blood loss (BL) (ml) and core body temperature (T) (°C) in all nine patients during each of the eight sampling periods in this study. The p_{O_2} and p_{CO_2} readings (mean values ± S.E.M.) from all nine patients taken at the post-graft sampling period are 165.9 ± 6.3 and 27.9 ± 0.9, respectively. Significant differences in mean values vs. pre-induction means were calculated by the two-tailed t-test: *, $p < 0.05$, **, $p < 0.02$, ***, $p < 0.01$, ****, $p < 0.001$.

As mentioned previously, the use of short columns and active filtering of the detector output lowered these detection limits to 5 pg of NE and E and 10 pg of DA in routine analysis.

Interferences

The following compounds were found not to interfere with the assay: L-dopa, α-methyl-dopa, dihyroxyphenylacetic acid, dihydroxyphenylglycol, 4-hydroxy-3-methoxyphenylglycol, noremetanephrine, metanephrine, ascorbic acid and uric acid.

Experimental

The mean values obtained for the plasma concentrations of NE and E, heart rate, mean arterial pressure (MAP), blood loss and core temperature for each sampling period are illustrated in Fig. 3.

As a preliminary test to establish the validity of subsequent *t*-test analyses, a univariate analysis of variance of repeated measures was performed [17]. From these tests, a Wilks lambda statistic was generated from which an F value was calculated. For NE, $F = 3.78$ ($p < 0.002$) and for E, $F = 7.87$ ($p < 0.0001$). Thus, the concentrations of both NE and E were changing significantly over the course of the measurement periods. In order to determine the periods in which significant changes occurred, individual *t*-tests were performed. Because the objective of the study was to determine whether or not the LC—ED method could determine the SNS response to surgical stress, catecholamine concentrations at each sampling period were compared to the pre-surgical values obtained at pre-induction. These results are presented below. All data presented were determined by LC—ED analysis on 5-μm particle size columns. A total of 22 samples were re-analyzed on 3-μm columns with dual-electrode detection. The calculated concentrations of catecholamines obtained from these determinations were all within 5% of the corresponding values obtained on 5-μm columns.

Norepinephrine

Plasma levels of NE were measured in a total of eight periods before, during, and after surgery. These data (Table II and Fig. 3) indicate that in seven out of nine patients, plasma NE concentrations tended to increase and plateau during the course of surgery above the baseline value of 332 ± 55 pg/ml measured prior to anesthesia induction. The mean increases in plasma NE concentration from post-induction through closure, failed to reach statistical significance ($p > 0.05$). The peak mean value recorded in the recovery room was significantly greater than baseline ($p < 0.02$).

Epinephrine

Baseline (pre-induction) plasma E concentrations and the changes observed during each sampling period for each patient are shown in Table III and Fig. 3. The plasma E concentration at pre-induction was 84 ± 18 pg/ml. The induction of anesthesia resulted in a significant depression in plasma E levels ($p < 0.05$ vs. pre-induction). The mean circulating plasma E concentration was then elevated above the mean pre-induction level during surgery, reaching

TABLE II

CHANGES IN PLASMA NOREPINEPHRINE CONCENTRATIONS (pg/ml) FROM PRE-INDUCTION DURING SURGERY

Patient	Pre-induction	Post-induction	Post-incision	Post-stripping	Post-graft	Post-decortication	Closure	Recovery room
1	505	+12	+204	+143	+189	+281	−17	+167
2	159	−3	+47	+100	+114	+88	+158	+127
3	528	−344	−156	−267	−69	−119	−266	−243
4	577	−253	+655	−29	−123	+21	−280	+334
5	275	−83	+302	+381	+340	+180	+209	+300
6	150	+108	+55	+67	+253	+361	+296	+728
7	204	+20	+42	+97	+244	+240	+375	+157
8	321	−110	−14	+273	+126	+29	−28	+261
9	270	−67	+250	+179	+29	+80	+147	+296
$\bar{x}$	332	−80	+154	+105	+123	+129	+66	+236
S.E.M.	55	47	78	61	51	50	77	84
p*		NS	NS	NS	NS	NS	NS	<0.02

*Versus pre-induction; NS = not significant.

TABLE III

CHANGES IN PLASMA EPINEPHRINE CONCENTRATIONS (pg/ml) FROM PRE-INDUCTION DURING SURGERY

Note that for statistical purposes, undetectable concentrations of E are converted to 45 pg/ml, the detection limit for E.

Patient	Pre-induction	Post-induction	Post-incision	Post-stripping	Post-graft	Post-decortication	Closure	Recovery room
1	96	−51	+288	−51	−51	+304	+323	+867
2	45	0	+3	+123	0	+97	0	+99
3	216	−171	−171	−158	−11	+527	+449	+340
4	45	0	0	+715	0	+596	0	+622
5	89	−44	+144	+64	+9	+54	+124	+219
6	81	−36	+141	+42	+220	+482	+540	+1352
7	45	0	+58	+216	+253	+59	+120	+195
8	96	−51	+357	+90	+22	+114	+57	+568
9	45	0	+28	+367	+261	+665	+964	+854
$\bar{x}$	84	−39	+94	+156	+78	+322	+286	+568
S.E.M.	18	18	53	86	42	83	107	136
p*		<0.05	NS	NS	NS	<0.01	<0.02	<0.001

*Versus pre-induction; NS = not significant.

statistical significance at post-decortication ($p < 0.01$), closure ($p < 0.02$) and in the recovery room ($p < 0.001$) where, like NE, the mean rise in plasma E levels reached its zenith (Fig. 3). Similar to NE, five out of nine patients showed consistent increases in plasma E during each surgical period. Of these five patients, four exhibited consistent increases in plasma NE levels as well. Of the remaining four patients, only two showed decreases in plasma E levels below baseline and the other two exhibited several periods of no change. All nine patients manifested increased levels of circulating E in the recovery room.

A comparison of the individual changes in the plasma concentrations of NE and E from pre-induction throughout the entire procedure (Fig. 4A) showed that changes in these two variables were weakly correlated ($r = 0.38, p < 0.01$). A stronger correlation between changes in E and NE (Fig. 4B) was observed in the recovery room ($r = 0.67, p < 0.05$).

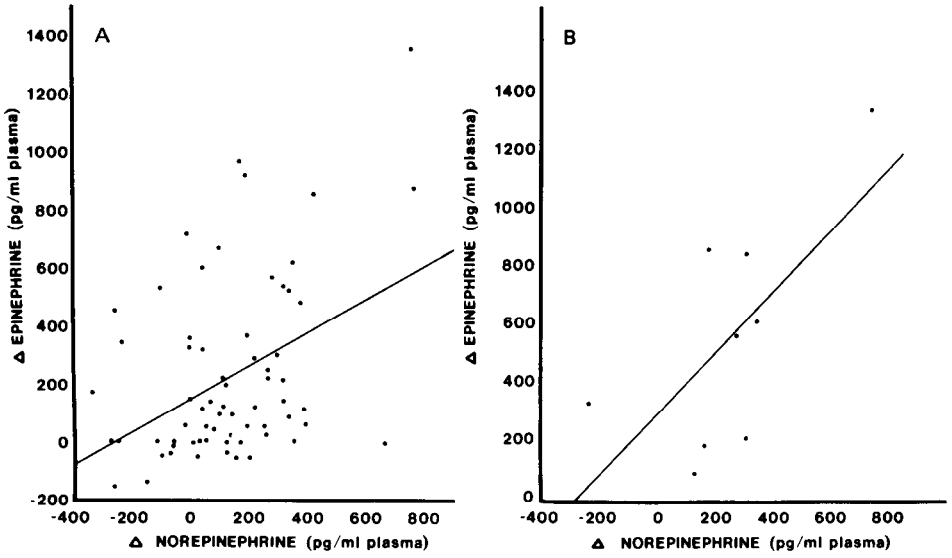

Fig. 4. Regression analyses of changes from pre-induction in the plasma concentrations of epinephrine (Δ epinephrine) vs. norepinephrine (Δ norepinephrine) in all nine patients. (A) During the entire study, $n = 63$; $y = 0.57x + 152$; $r = 0.38$; and $p < 0.01$. (B) In the recovery room, $n = 9$; $y = 1.08x + 312$; $r = 0.68$; and $p < 0.05$.

Blood pressure, heart rate and blood loss

The changes in mean arterial pressure (MAP) and heart rate, from the pre-induction stage throughout the procedure, are shown for each patient in Table IV. Blood loss during each sampling period is illustrated in Fig. 3 and Table I. Only one of the first two variables, MAP, changed significantly and this occurred only during the post-decortication period (mean decrease ± S.E.M.: 10 ± 4 mm; $p < 0.05$). Linear regression analyses showed no significant correlation between changes in concentration of either catecholamine and changes in MAP, heart rate or absolute blood loss per stage. Furthermore, no correlation was observed between changes in MAP and changes in heart rate, or between absolute blood loss per stage and heart rate. A slight, but significant negative correlation was observed between changes in MAP vs. absolute blood loss per stage during the surgical periods where bleeding occurred ($r = -0.31$, $p < 0.02$). This relationship is illustrated in Fig. 5A.

Core temperature

Table IV shows the changes in core temperature observed in each patient. Significant decreases in mean body temperature were observed at post-incision ($p < 0.05$), post-stripping, post-graft, post-decortication (all measurements, $p < 0.01$) and at closure ($p < 0.05$). Mean body temperature returned to the pre-induction level in the recovery room. Linear regression analyses of changes in body temperature versus changes in NE and E concentrations revealed a weak positive correlation in both comparisons (body temperature vs. NE: $r = 0.25$, $p < 0.05$; body temperature vs. E: $r = 0.28$, $p < 0.05$). These relationships are illustrated in Fig. 5B and C.

TABLE IV

CHANGES IN MEAN ARTERIAL PRESSURE (MAP, mmHg) HEART RATE (HR, beats/min) AND CORE TEMPERATURE (T_b, °C) FROM PRE-INDUCTION VALUES DURING SURGERY

Patient	Parameter	Pre-induction	Post-induction	Post-incision	Post-stripping	Post-graft	Post-decortication	Closure	Recovery room
1	MAP	77	+3	+3	−2	−6	−4	+7	0
	HR	72	+8	−12	−10	−8	−8	+10	+20
	T_b	36.0	−0.2	−0.5	−0.5	−0.5	−0.5	−0.2	+0.3
2	MAP	87	+5	−9	−2	+3	−8	−11	−2
	HR	80	−2	0	+2	−2	+30	+50	+26
	T_b	36.0	−0.8	−1.8	−1.9	−2.2	−4.1	−5.0	+0.6
3	MAP	73	−9	+5	−2	−4	+3	+26	+9
	HR	90	−6	−24	−26	−29	−26	+1	−10
	T_b	36.5	0	−0.3	−2.3	−2.5	−2.5	−2.5	−2.5
4	MAP	93	−15	−3	−20	−23	−30	−10	−20
	HR	100	−20	−10	−20	−24	−20	−30	−28
	T_b	37.5	−0.1	−0.5	−0.6	−0.9	−0.9	−0.6	−0.5
5	MAP	95	−36	−27	−13	−28	−28	+13	−15
	HR	52	+8	+18	+26	+12	+12	+7	+28
	T_b	36.0	0	−0.1	−0.1	−1.5	−1.5	−1.5	+1.0
6	MAP	62	+6	−7	+3	−2	−2	+23	+11
	HR	70	+24	−13	+1	+4	0	+34	+5
	T_b	36.5	0	−0.3	−0.4	−0.6	−0.2	+0.7	+1.0
7	MAP	84	−21	−12	−7	+1	−7	−4	−18
	HR	78	−2	−14	−10	−14	−20	−22	−4
	T_b	36.5	−0.7	−1.5	−1.5	−1.5	−1.4	−1.4	−0.9
8	MAP	74	+11	+15	+11	+11	−14	+11	+19
	HR	76	−10	−19	−11	−9	−16	−16	+12
	T_b	36.0	0	−1.0	−1.4	−1.5	−1.8	−1.8	0
9	MAP	67	−10	+8	−1	+23	−2	−1	+7
	HR	68	+14	+13	−3	−5	−7	−8	+10
	T_b	36.0	−0.3	−0.5	−0.5	−0.7	−0.7	−0.1	+0.9
$\bar{x}$ ± S.E.M.	MAP	79 ± 4	−7 ± 5	−3 ± 4	−4 ± 3	−3 ± 5	−10 ± 4	+6 ± 4	−1 ± 5
	HR	76 ± 4	+2 ± 4	−7 ± 5	−6 ± 5	−8 ± 4	−6 ± 6	+1 ± 9	+7 ± 6
	T_b	36.2 ± 0.1	−0.2 ± 0.1	−0.7 ± 0.2	−1.0 ± 0.3	−1.5 ± 0.4	−1.5 ± 0.4	−1.4 ± 0.6	0 ± 0.4
p*	MAP		NS	NS	NS	NS	<0.05	NS	NS
	HR		NS	NS	NS	NS	NS	NS	NS
	T_b		NS	<0.05	<0.01	<0.01	<0.01	<0.05	NS

* Versus pre-induction; NS = not significant.

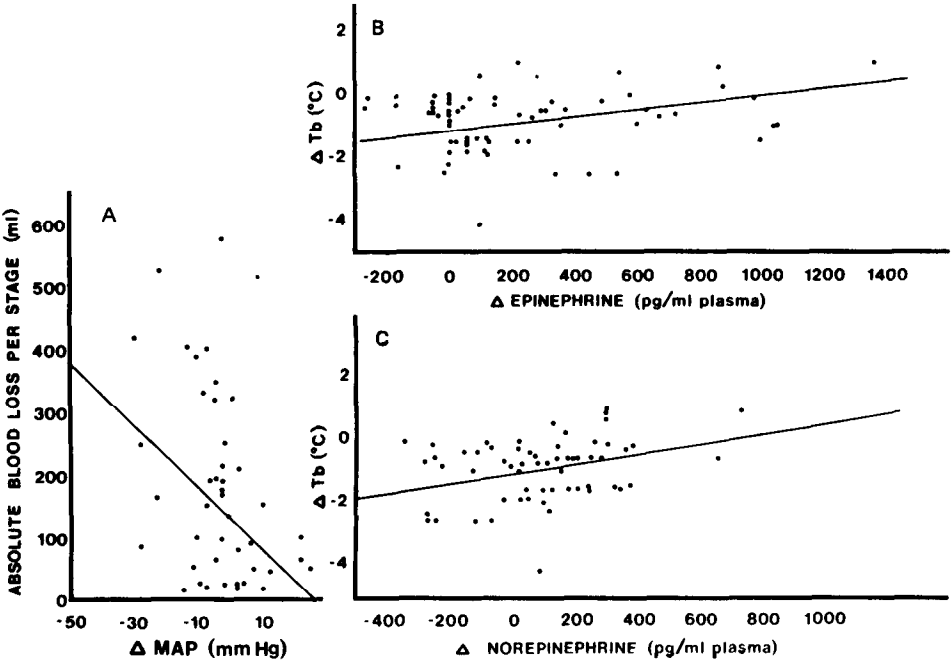

Fig. 5. Regression analyses of: (A) absolute volume of blood lost at each period from post-incision to closure vs. corresponding changes in mean arterial pressure from pre-induction values (△ MAP), $n = 45$, $y = -3.3x + 133$, $r = -0.31$ and $p < 0.02$; (B) changes in core body temperature from pre-induction (△ T_b) vs. changes in the plasma concentrations of epinephrine (△ epinephrine) during the entire study, $n = 63$, $y = 0.0010x - 1.12$, $r = 0.28$; and (C) △ T_b vs. △ norepinephrine during the entire study, $n = 63$, $y = 0.0014x - 1.02$, $r = 0.26$ and $p < 0.05$.

Blood gases

Arterial blood gases were monitored during the post-graft sampling period (Fig. 3 and Table I). The results indicate that each patient was adequately ventilated and oxygenated. Linear regression analyses of the absolute levels of pO_2 and pCO_2 versus the changes in plasma catecholamine concentration from pre-induction to post-graft revealed no significant correlation between any of these parameters.

DISCUSSION

The LC—ED method described was used to determine the degree of sympathetic nervous system activation obtained during the course of spinal fusion surgery for the correction of scoliosis and to compare these results to those obtained during abdominal surgery using a radioenzymatic method [11]. While the degree of stress associated with each surgical procedure may be different, and the anesthesia protocol during these procedures was different, the results obtained with each assay procedure were comparable. Baseline (pre-anesthesia induction) concentrations of NE in both studies were similar to those seen in normal resting, unanesthetized subjects [22—25], while E concentrations were slightly elevated [24, 25]. The induction of anesthesia

resulted in a significant fall in E, but not NE concentrations in both studies as well.

On the other hand, none of our five intraoperative NE values were significantly different from baseline (although clearly elevated) while the one intraoperative measurement of NE reproted by Halter et al. [11] was significantly greater than baseline. In contrast, the increases in plasma E levels seen in both studies were comparable.

Finally, the greatest intraoperative increases in the plasma concentrations of both catecholamines were seen late in surgery (during closure in our study and during extubation in the study of Halter et al. [11]) and in the recovery room, in both studies. Thus, the only major difference seen in the two studies concerns the noradrenergic response to surgery. If the conclusion of Halter et al. [11], that the SNS response to surgery is mediated by afferent pain information, is correct, the inclusion of the opioid narcotic fentanyl in our study could account for the diminished noradrenergic response.

There are several other factors besides afferent pain signals that could account for the intraoperative changes in catecholamine concentration we observed. One factor might be the stress associated with pre-surgical anxiety experienced by the young patients in this study. Wherease pre-induction plasma NE concentrations in our subjects were very similar to those seen in normal, resting subjects [22—25], the pre-induction plasma E concentrations were clearly elevated in our patients [24, 25]. These elevations were insignificant compared to the elevations in plasma E levels seen during surgery and in the recovery room, however. Furthermore, the induction of anesthesia resulted in a significant drop in plasma E levels indicating that anxiety is not a factor under general anesthesia.

In agreement with Halter et al. [11] we found that the induction of anesthesia resulted in lower plasma catecholamine concentrations, particularly in E concentrations. Thus, it is unlikely that any of the drugs employed in the induction or maintenance of anesthesia caused SNS activation. Consistent with this observation is the significant rise in plasma NE and E levels observed in the recovery room, when the effects of anesthesia were subsiding.

Arterial hypotension is also a known cause of SNS activation [26]. It is thus possible that hypotensive episodes in these patients resulted in increased catecholamine release. However, MAP decreased significantly below baseline only during post-decortication, possibly due to a transient hypovolemia caused by blood loss during this and the proceeding period (see Fig. 5A). Whereas mean plasma E concentrations were significantly elevated above baseline during this period, subsequent significant increases in E levels at closure, and in both NE and E levels in the recovery room, were not associated with a lowered MAP or significant blood loss. Furthermore, if hypotension was a major factor in the observed SNS activation, one would expect to see a negative correlation between changes in MAP and catecholamine concentrations or a positive correlation between blood loss and catecholamine changes. Our evidence does not support this. Thus, while hypotension may have contributed to the increased plasma E levels recorded during the post-decortication period, our data indicate that it cannot account for SNS activation at other periods. Furthermore, because no significant changes in heart rate occurred, it is not likely that this was a factor in the observed SNS activation.

Hypothermia is another possible cause of increased catecholamine release [26]. Mean internal body temperature was indeed significantly lowered from post-incision to closure in this study. Lowered body temperature during general anesthesia, however, is caused by the suppression of the CNS thermoregulatory mechanism [27], and is not a true hypothermia. Therefore, activation of heat production/conservation responses would be delayed until after the suppressive effects of general anesthesia were removed. The weak but significant positive correlation that existed between changes in body temperature and changes in NE and E indicates that the lowered body temperature measured under general anesthesia may actually suppress SNS outflow slightly (see Fig. 5A and B). Thus, the lack of a negative correlation between these variables and the lack of a negative relationship between decrements in core temperature and plasma catecholamine levels in the two patients with the largest decreases in core temperature (patients Nos. 2 and 3, see Tables II—IV), clearly indicate that SNS activation during surgery was not temperature-induced.

Following the termination of anesthesia, recovery room measurements of core temperature, plasma NE and E showed large increases. The CNS thermoregulator, no longer suppressed by anesthesia, responded to low core temperature (at this point, a true hypothermia) with a strong activation of heat production/conservation responses, which include increases in SNS outflow [28]. It is therefore possible that the increase in mean plasma catecholamine concentration observed in the recovery room was, in part, related to the recovery from low body temperature.

Hypoxia and/or hypercarbia can also cause SNS activation [26]. Arterial blood gas measurements at post-graft, however, revealed no abnormalities in either pO_2 or pCO_2 and indicated that oxygenation and ventilation were satisfactory. Furthermore, no correlation was observed between the absolute levels of pO_2 or pCO_2 vs. the changes in plasma catecholamines from baseline to the post-graft sampling period.

Thus, while it is possible that episodic hypotension during surgery and hypothermia following surgery could play a role in the SNS activation observed in this study, their long-term contribution is not a major one. The lack of consistent or obvious effects during surgery of various hemodynamic factors (MAP, heart rate, blood loss) blood gas changes, and low body temperature on plasma catecholamine concentrations is compatible with the conclusion of Halter et al. [11], that SNS activation during surgery is primarily a response to afferent pain signals from the site of trauma. In this light, it is reasonable to assume that the inclusion of opiate or opioid agents as part of anesthesia management can obtund SNS responsiveness. In fact, Stanley et al. [29] have shown that fentanyl can diminish plasma catecholaminergic elevations, particularly NE, during coronary artery surgery. This might explain the fact that while Halter et al. [11] saw a strong correlation between changes in NE and E during surgery, we observed the strongest correlation in these parameters in the recovery room, when the effects of fentanyl had subsided (see Fig. 4A and B). We conclude that the lesser increase in plasma NE intraoperatively in this study as compared to the study of Halter et al. [11] is not related to different assay techniques, but rather to differing anesthesia management protocols.

In summary, the LC—ED method described offers many advantages over the radioenzymatic assay for plasma catecholamines. The greater ability to screen out potential interferences with LC—ED and its overall simplicity and rapidity make it an ideal choice in a clinical setting. LC—ED is particularly appropriate in cases where sample volume is not limiting and where plasma catecholamine concentrations, particularly DA [17], are elevated. In these cases, the greater sensitivity of radioenzymatic procedures is not a factor. It is likely, that the continuing improvement in chromatographic column technology will obviate the present sensitivity advantages of radioenzymatic methods.

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