\(\beta_2\)-adrenergic responsiveness in vivo during abdominal surgery

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Summary

We have studied adrenergic function in vivo during anaesthesia and surgery. Epinephrine 50 ng kg\(^{-1}\) min\(^{-1}\) was given by i.v. infusion over 30 min to 10 healthy adult volunteers and to 10 patients undergoing abdominal operations. The cAMP response to stimulation by epinephrine, which was obtained as the area under the curve (AUC) for plasma cAMP concentration divided by the AUC for plasma concentration of epinephrine, was more pronounced during surgery (mean ratio 3.5) than in the control situation (ratio 1.4; \(P<0.02\)). This resulted in greater hypokalaemic and hyperglycaemic responses (ratios –0.67 and 4.5) than in the control group (ratios –0.33 and 1.6, respectively; \(P<0.004\)). Mean arterial pressure decreased in the control group while it increased in the study group, and serum cortisol concentration was higher in those who underwent surgery (\(P<0.02\)). These results are consistent with an increased adrenergic response during abdominal surgery. (Br. J. Anaesth. 1998; 81: 343–347).

Keywords: surgery, abdominal; surgery, adrenergic response; blood, glucose; sympathetic nervous system, epinephrine; cardiovascular system, response; receptors, adrenergic.

The adrenergic response, which is of paramount importance for physiological reactions during surgery, is dependent on the function of several molecular mechanisms.1,4 The \(\beta\)-receptor operates by binding an agonist at the cell surface, which activates a specific G-protein which, in turn, catalyses the conversion of ATP to cyclic adenosine monophosphate (cAMP). This regulator of cell function alters the balance of phosphorylation that promotes specific adrenergic effects. Mechanisms for reducing the response include uncoupling of the \(\beta\)-receptor from the G-protein, increase in the distance between these molecular complexes (sequestration) and reduction in the number of receptors (down-regulation).1,2

Attenuation of the adrenergic response (desensitization) is known primarily to occur during adrenergic stimulation,2,3 which is of interest as plasma concentrations of catecholamines (primarily epinephrine) become increased during surgery. Exhaustion has been confirmed in vitro by a reduction of the \(\beta\)-adrenergic receptor density on lymphocytes4 and by reduced cAMP production on stimulation with isoproterenol,5 while increased responsiveness has also been reported.6 Fewer attempts have been made to measure \(\beta\)-adrenergic responsiveness in vivo.

In this study, we have looked for a set of variables based on blood chemistry and physiological reactions that are suitable for quantification of \(\beta\)-adrenergic responses during surgery. For this purpose, integrated time-effect profiles7 were used to compare adrenergic agonist pressure with variables known to be affected by \(\beta\)-adrenergic receptor stimulation in vivo in a group of patients undergoing abdominal operations compared with a control group. Epinephrine was used as the adrenergic agonist as the purpose was to evaluate the sum of exogenous and endogenous \(\beta\)-stimulation. Serial measurements of the concentration of free cAMP in plasma were made as this variable indicates the level between receptor and effect at which any difference in adrenergic responsiveness is located.

Patients and methods

An i.v. infusion of epinephrine, dissolved in 500 ml of normal saline, was given at a rate of 50 ng kg\(^{-1}\) min\(^{-1}\) over 30 min (total dose approximately 0.1 mg) to 10 patients aged 34–56 (mean 45) yr undergoing abdominal surgery. The operations were colectomy (\(n=2\)), hysterectomy (\(n=4\)), resection of ovarian cysts (\(n=3\)) and pancreatoduodenectomy (Whipple’s operation, \(n=1\)). The same infusion was also given to 10 healthy volunteers (aged 31 (range 23–44) yr). All subjects were free from cardiovascular and pulmonary disease and were not receiving regular medications. The study was approved by the Ethics Committee of Huddinge University Hospital and all subjects gave informed consent.

All subjects were studied after fasting for at least 6 h. Premedication comprised morphine 7.5–10 mg i.m., approximately 1 h before arriving in the operating theatre. Anaesthesia was induced with thiopental and maintained with fentanyl 0.15–0.45 (mean 0.29) mg, and 1–3% isoflurane and oxygen in ambient air, as required. Oxygen saturation (pulse oximetry) and end-tidal carbon dioxide concentration were monitored continuously, the latter being maintained at 4.5–5.0 kPa. During surgery and the experiment, i.v. fluid supplementation comprised normal saline 4.0–7.0 (mean 4.9) ml kg\(^{-1}\) h\(^{-1}\). Warm air blankets and warmed infusions were used to maintain body temperature within 37 ± 1°C, confirmed by
oesophageal temperature monitoring in half of the patients. The volunteers underwent the infusion experiment with epinephrine but received no other drug and did not undergo surgery.

Blood samples were obtained via a cannula placed in a cubital vein of the arm not used for infusion, immediately before the infusion started, and repeatedly over 100 min. In the surgical patients, the infusion was started immediately after skin incision. Specimens for measurement of plasma concentrations of epinephrine and cAMP were placed in ice water and centrifuged within 30 min. The remaining plasma was stored at −70 °C until analysed.

Plasma concentrations of adrenaline were measured by high pressure liquid chromatography. Plasma concentrations of free cAMP were measured by ion-pair reversed-phase chromatography. The free fraction is probably the most sensitive indicator of cAMP leakage from cells, although some of the cAMP also binds to plasma proteins. These analyses were performed with a coefficient of variation of 2%.

Blood glucose was measured using a colorimetric method (Johnson and Johnson Ektachem, Rochester, MI, USA), plasma cortisol by ELISA (Enzymun-Test, Boehringer Mannheim GmbH, Ingelheim, Germany) and plasma insulin by radioimmunoassay (Pharmacia RIA 100, Uppsala, Sweden). Serum potassium concentration was measured by a potentiometric test (Johnson and Johnson Ektachem). The coefficients of variation for these analyses were 4.6%, 5%, 6% and 4%, respectively. Confounding factors inherent in the sampling and analysis of serum potassium were avoided, including the use of a tourniquet, succinylcholine, muscle activity and haemolysis.

Heart rate and arterial pressure were measured every 5 min using a Datex AS 3 (Datex, Helsinki, Finland) or an HP 56S (Hewlett Packard Co., MA, USA). Mean arterial pressure (MAP) was calculated as the sum of diastolic pressure and one-third of the difference between systolic and diastolic pressures.

Glucose and insulin data from one volunteer were excluded because of suspected reduction of the cellular sensitivity to insulin. Her baseline insulin concentration was more than 10 times higher than the mean value for the other subjects.

Results are reported as mean (SD). When there was skewed distribution, median and range were used. Measurements that were performed frequently were also expressed as area under the curve (AUC) using the linear trapezoidal method. The half-life of epinephrine was estimated from the last three points in time using a monoexponential elimination function. Differences between treatments were evaluated by analysis of variance (ANOVA) or the Mann–Whitney test, as appropriate. Correlations were tested by simple and multiple linear regression analysis. Differences were reported to be significant at P<0.05.

### Results

Infusion of epinephrine increased plasma concentrations of epinephrine, cAMP and glucose, and decreased serum potassium concentration (fig. 1). Changes over time for epinephrine, as measured by AUC, were slightly, but not significantly, higher in the control group than in those who underwent surgery (table 1). The reason became evident in our pharmacokinetic analysis, which showed that the terminal half-life of epinephrine was shorter during surgery (median 14 (range 6–28) min compared with 46 (17–122) min) (Mann–Whitney, P<0.001). However, CAMP and blood glucose responses to epinephrine were greater and serum potassium concentration tended to be more depressed in patients than in volunteers (table 1).

Change in serum potassium was the index of β-adrenergic stimulation that correlated most closely with AUC for plasma epinephrine (table 2; fig. 2, left). AUC for the decrease in serum potassium concentration also correlated with the magnitude of a further increase in plasma epinephrine concentration that occurred during the first 10 min after infusion (r=−0.68, P<0.001; fig. 1, upper left).

The ratios between AUC values were calculated to determine if any difference in β-adrenergic effect could be attributed to changes in the responsiveness to the first (epinephrine) or second (cAMP) messenger systems. This analysis showed that surgery was followed by significantly more pronounced changes in blood variables (cAMP, serum potassium and blood glucose) in response to infusion of epinephrine. In contrast, serum potassium and blood glucose responses to cAMP were the same in both groups (table 1).

As expected, heart rate increased when epinephrine was given. In contrast with all other indices of adrenergic response, the heart rate response to cAMP was greater in the control group than in the study group (table 3). MAP increased in the study group (table 3).

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Abdominal surgery</th>
<th>ANOVA</th>
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<tbody>
<tr>
<td><strong>AUC</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>∆P-epinephrine (nmol litre⁻¹ min⁻¹)</td>
<td>83.2 (27.4)</td>
<td>61.6 (33.1)</td>
<td>P&lt;0.005</td>
</tr>
<tr>
<td>∆P-cAMP (nmol litre⁻¹ min⁻¹)</td>
<td>102.1 (36.5)</td>
<td>165.7 (51.4)</td>
<td></td>
</tr>
<tr>
<td>∆S-K (nmol litre⁻¹ min⁻¹)</td>
<td>−26.7 (12.1)</td>
<td>−38.4 (19.5)</td>
<td></td>
</tr>
<tr>
<td>∆B-glucose (nmol litre⁻¹ min⁻¹)</td>
<td>112.1 (30.8)</td>
<td>215.1 (114.9)</td>
<td>P&lt;0.02</td>
</tr>
<tr>
<td><strong>AUC ratios</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>∆P-cAMP / ∆P-epinephrine</td>
<td>1.4 (1.0)</td>
<td>3.5 (2.3)</td>
<td>P&lt;0.02</td>
</tr>
<tr>
<td>∆S-K / ∆P-epinephrine (10⁴)</td>
<td>−0.33 (0.12)</td>
<td>−0.67 (0.26)</td>
<td>P&lt;0.002</td>
</tr>
<tr>
<td>∆S-K / ∆P-cAMP (10⁴)</td>
<td>1.6 (0.9)</td>
<td>4.5 (2.7)</td>
<td>P&lt;0.004</td>
</tr>
<tr>
<td>∆B-glucose / ∆P-cAMP (10⁴)</td>
<td>1.3 (0.6)</td>
<td>1.4 (0.8)</td>
<td></td>
</tr>
</tbody>
</table>
Adrenergic function during surgery

The function of the β-adrenergic receptor is often assumed to be depressed during surgery because of the effects of anaesthesia and intrinsic preoperative and intraoperative adrenergic stimulation. Such conclusions are usually based on in vitro stimulation of cAMP production or on measurements of receptor density and binding sites on leucocytes.4 5 Physiological variables reflecting adrenergic responsiveness in the whole organism have occasionally been used in volunteer experiments10 but not during surgery.

In this study, we evaluated responsiveness in vivo, from relationships between blood chemistry and haemodynamic state during adrenergic stimulation. Cyclic AMP resulting from adrenergic activation leaks out from cells and, although some breakdown occurs, plasma concentrations of cAMP and epinephrine correlate well in volunteers11 and during surgery.12 Therefore, the cAMP response was used as evidence of desensitization of the β-adrenergic receptor complex, while attenuation of the effects of epinephrine on serum potassium, blood glucose and heart rate indicated block of either the first or second messenger.13–15

Discussion

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The choice of adrenergic agonist for β₂ activation was crucial. Epinephrine was considered more suitable than a β₂-selective agonist as increased release of epinephrine from endogenous sources is part of the “stress” response to surgery. The use of epinephrine allows reasonable comparisons to be made between β₂ agonist pressure (here represented by the AUC for epinephrine in plasma) and physiological responses. In contrast, the use of a β₂-selective agonist would require that our value for agonist pressure considered the AUC for both infused β₂-selective agonist and endogenous epinephrine, which is a speculative approach. Epinephrine is not the ideal agonist, however, as it stimulates both β₂ and α receptors. Therefore, the dose was adjusted to generate predominantly β₂ effects.¹⁶ ¹⁷

The results suggest that the responsiveness of the β₂-adrenergic receptor is increased, rather than decreased, during the first 80 min of abdominal surgery. This conclusion is based on the fact that the cAMP response was stronger for any concentration–time profile of epinephrine when surgery was performed. AUC ratios also showed that the hypokalaemic and hyperglycaemic responses to epinephrine were stronger during surgery. These differences can be attributed to the increased cAMP effect, as AUC ratios showed that the hypokalaemic and hyperglycaemic responses relative to cAMP stimulation were similar in both groups.

The marked increase in heart rate in the control group was probably a result of reflex tachycardia associated with vasodilatation in skeletal muscle resulting from stimulation of β₂-receptors. This view is supported by simultaneous reduction of mean arterial pressure, which was caused by a decrease in diastolic pressure, and by the high heart rate/cAMP ratio. In patients who underwent surgery, however, noradrenergic and sympathetic mechanisms probably contributed to more widespread activation of α receptors, which increased arterial pressure. Therefore, the increase in heart rate became less pronounced. An α effect in the patient group was also evidenced by depression of serum insulin concentration during infusion¹³ while the sharp increase at the end of the study probably represented adjustment to high blood glucose concentration.

It may also be important that heart rate was much higher at the start of surgery compared with baseline

![Figure 2](image_url) Area under the curve (AUC) for changes in the concentrations of serum potassium (K) and plasma epinephrine (left) and for blood glucose and serum insulin concentrations (right), during and after infusions of epinephrine. One datum point represents one experiment.
Adrenergic function during surgery

conditions in volunteers. One may assume that cAMP stimulation results in a greater increase in heart rate starting from a baseline pulse of 65 beat min\(^{-1}\) compared with 90 beat min\(^{-1}\). The difference in heart rate can be regarded as the result of preoperative stress, which increases plasma epinephrine concentration, and tracheal intubation, which is known to have the same effect.

Our data are consistent with an in vivo study by Tøhmer and Cryer\(^{1}\) who suggested that adrenergic responsiveness is increased 30–60 min after the onset of surgery while it is reduced after 4–6 h of surgery. Other in vitro\(^{5,6}\) and in vivo\(^{11}\) studies suggest that adrenergic responsiveness is reduced early during surgery. Löfgren and Hahn showed that absorption of epinephrine added to local anaesthetics used for intercostal nerve block immediately after open cholecystectomy was associated with the same responsiveness as in a control situation, despite the fact that plasma epinephrine concentrations were much higher in patients.\(^{14}\)

Serum potassium concentration appeared to be the variable that correlated best with the degree of adrenergic stimulation, as indicated by the AUC of indices of 2-adrenergic receptor function.\(^{15}\) Serum potassium concentration was measured after considering potential confounding factors, such as physical activity.\(^{16}\)

It does not matter much that baseline values differed slightly as only changes were used in the calculations. Epinephrine reduces serum potassium concentration by increasing the uptake of potassium in skeletal muscle via activation of Na–K-ATPase in cell membranes. The effect is mediated by β\(_2\)-receptors and is not obtained with isoproterenol, a catecholamine often used for studies of adrenergic responsiveness.\(^{16}\)

Two factors should be considered when evaluating the results of our study. In both volunteers and patients, there was a paradoxical increase in epinephrine responsiveness with age,\(^{20}\) the difference in age between the groups cannot explain the increased responsiveness found. The shorter half-life of epinephrine in patients may also be a result of other factors—plasma clearance of epinephrine is known to decrease by approximately 20% in old age, and the epinephrine response to stress is reduced.\(^{21}\)

A more likely cause is increased peripheral uptake of the hormone as part of the “stress” response to surgery.

In summary, we have found that β\(_2\)-adrenergic responsiveness measured in vivo was increased slightly during the first 80 min of abdominal surgery. This does not preclude the fact that desensitization of β-receptors may occur in more prolonged operations.

References