Effects of naloxone on catecholamine plasma levels in adult men.
A dose-response study

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Abstract. To evaluate a possible role for endogenous opiates in modulating sympathetic-adrenal function in humans, we measured plasma epinephrine and norepinephrine (radioenzymatic method), blood pressure and heart rate in 8 normal men (aged 24–33 years) before and after placebo or different doses (0.4, 4.8, 10 mg) of naloxone. In 6 subjects plasma insulin and glucagon levels were also measured by radioimmunoassay after placebo and 10 mg naloxone.

Naloxone had no significant effect upon blood pressure, heart rate, plasma insulin, glucagon or norepinephrine. Placebo, 0.4 and 4.8 mg naloxone caused no significant change in peripheral levels of epinephrine while 10 mg produced an increase in epinephrine concentrations 15 min after iv injection (186 ± 23 vs 99 ± 9 pmol/l, P < 0.01).

Since naloxone did not modify plasma levels of insulin and glucagon, an indirect effect of naloxone on adrenal medullary secretion seems to be excluded.

These results are in agreement with in vitro experimental data obtained in animals and suggest that endogenous opiates also have a role in modulating adrenal medullary secretion in man.

Various reports indicate that opiate peptides are present not only in brain structures (Hughes et al. 1975; Smith et al. 1976; Yang et al. 1977) but also in peripheral tissues (Hughes et al. 1977; Schultzberg et al. 1978; Di Giulio et al. 1980) such as the adrenal medulla and sympathetic ganglia. The physiological role(s) of opiate peptides in these structures is still uncertain but some authors suggest that opiates may act as modulators of sympathetic-adrenal activity.

In vivo experiments using morphine administration have led to controversial results (Taborsky et al. 1981). Morphine has been shown to increase, decrease or not affect plasma catecholamines (CA) in dogs, depending on the dose and on the experimental conditions. Similarly, in patients suffering acute myocardial infarction, morphine administration has been reported to cause an increase, decrease or no change in blood pressure (BP) or heart rate (HR) (Gould et al. 1978).

In contrast, in vitro experiments are less controversial. Konishi et al. (1979) reported that enkephalins inhibit cholinergic transmission in the sympathetic ganglia of guinea pigs and Kumakura et al. (1980) observed that nicotine-stimulated release of CA from isolated bovine chromaffin cells can be reduced by opiate agonists. The effect was reversed by naltrexone.

These in vitro experiments seem to support an inhibitory role for opiates in peripheral sympathetic function.

Since naloxone is a competitive antagonist of opiate receptors, any change induced by its administration may be taken as convincing evidence for a physiological involvement of opiate receptors in the control of sympathetic-adrenal activity.

To evaluate a possible role for endogenous opiates in modulating sympathetic-adrenal function in
humans, we measured plasma epinephrine (E) and norepinephrine (NE), BP and HR in 8 normal volunteers after iv administration of saline or different doses of naloxone (0.4, 4.8, 10 mg). In 6 subjects plasma insulin and glucagon levels were also measured after placebo and 10 mg naloxone.

Materials and Methods
The study was performed on 8 normal men aged 24–33 years and was single (patient) blind. Informed consent was obtained from all subjects. Each subject was tested in random order on 4 different days, at least 5 days apart. All experiments were performed after an overnight fast, between 08.00 and 09.00 h and after at least 12 h of non-smoking.

On the experimental day an antecubital vein was cannulated and kept patent with 0.9% saline. After at least 30 min of quiet recumbency, a basal blood sample was drawn, followed immediately by a 1 min iv injection of 12 ml of saline or different doses of naloxone (naloxone hydrochloride, Endo Laboratories, Inc., New York, USA). Further blood sampling was carried out 15 and 30 min after naloxone administration.

The time course of the experiment was based on the observation that in a phaeochromocytoma patient naloxone administration caused an elevation of CA levels 10–15 min after injection (Mannelli et al. 1983).

Samples for CA measurement were drawn in vacuum containers containing 100 µl of a solution with glutathione (60 mg/ml) and EGTA (90 mg/ml), put immediately in an ice-water bath and centrifuged at 4°C. Plasma samples were stored at ~70°C until assayed.

Plasma samples for glucagon measurement were drawn in cold tubes containing 1000 IU TrasyloL™ and 2 mg EDTA-Na2/ml blood, were centrifuged at 4°C and stored frozen until assayed.

All specimens (control and experimental samples) were measured in duplicate in the same assay.

Plasma CA were assayed by a radioenzymatic method previously described (Neri Serneri et al. 1981). The radioenzymatic method used yielded 27 cpm/pg E and 23 cpm/pg NE with blank values of 11–27 (mean 20) cpm for E and 14–32 (mean 24) cpm for NE. Since 50 µl of plasma was used for each assay, about 15 pg/ml (82 pmol/l) of E and 20 pg/ml (118 pmol/l) of NE yielded cpm values double those of the blanks. Therefore these concentrations can be taken as the limit of assay sensitivity for E and NE, respectively.

Glucagon levels were measured by radioimmunoassay using reagents furnished by Biodata (Milan, Italy). The antiserum used was obtained in rabbits immunized according to the method of Assan et al. (1965) and the working dilution was 1:8000. The cross-reaction of the antiserum with glucagon-like peptides of enteric origin was tested using human gastro-intestinal extracts and was absent at up to 50 mg of tissue/ml. A solution of poly-ethyleneglycol was employed for the separation technique. The sensitivity of the method was 15 pg/ml.

<table>
<thead>
<tr>
<th>Time</th>
<th>Placebo</th>
<th>0.4 mg</th>
<th>4.8 mg</th>
<th>10 mg</th>
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<tr>
<td>Heart rate</td>
<td></td>
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</tr>
<tr>
<td>0</td>
<td>62.8 ± 1.8</td>
<td>64.3 ± 3.9</td>
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<td>+ 15</td>
<td>62.8 ± 2.0</td>
<td>64.3 ± 3.0</td>
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<tr>
<td>+ 30</td>
<td>61.7 ± 2.5</td>
<td>64.0 ± 2.2</td>
<td>64.8 ± 2.8</td>
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<td>Systolic BP</td>
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<td></td>
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<tr>
<td>0</td>
<td>111.4 ± 3.9</td>
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<td>116.4 ± 4.3</td>
<td>107.2 ± 3.0</td>
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<tr>
<td>+ 15</td>
<td>107.5 ± 4.8</td>
<td>113.6 ± 2.4</td>
<td>115.0 ± 5.1</td>
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<tr>
<td>+ 30</td>
<td>107.8 ± 4.3</td>
<td>105.4 ± 3.9</td>
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<td>Diastolic BP</td>
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<tr>
<td>+ 15</td>
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<tr>
<td>+ 30</td>
<td>73.6 ± 4.8</td>
<td>68.6 ± 3.0</td>
<td>75.0 ± 2.4</td>
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Plasma insulin was measured by radioimmunoassay according to the method of Hales & Randle (1963), using reagents furnished by Sorin (Saluggia, Italy).

BP was measured by a Riva-Rocci sphygmomanometer. Each value is the mean of three consecutive measurements.

Statistical analysis was performed using a t-paired test corrected for the repeated use. All data are expressed as mean ± SEM.

Results

Mean (± SEM) HR, systolic and diastolic BP are reported in Table 1. No significant changes in HR or BP were observed after placebo or naloxone administration.

Insulin and glucagon circulating levels were also unchanged after placebo or 10 mg naloxone. These results are reported in Table 2.

Mean (± SEM) E plasma levels are shown in Fig. 1 and Table 3. Placebo and 0.4 mg naloxone did not induce any change; 4.8 mg naloxone caused an increase which was not significant while 10 mg naloxone caused a significant increase in E plasma levels after 15 min (99 ± 9 vs 186 ± 23 pmol/l, P < 0.001). The increase was observed in each subject studied.

Naloxone, in contrast was not able to induce any significant change in NE plasma levels in doses up to 10 mg (Table 4).

Discussion

The hypothesis that sympathetic-adrenal activity is modulated by endogenous opiates is still under debate.

In vivo experiments performed with opiate agonist administration led to controversial results (Taborsky et al. 1981; Yukimura et al. 1981; Petty et al. 1981; Van Loon et al. 1981) probably because of the dose-related side effects elicited by these substances. These unpleasant side effects can in fact produce a stressful condition in which the
possible modulatory role of opiates is overwhelmed by activation of the sympathetic nervous system.

Therefore, to evaluate in vivo the involvement of opiate receptors in modulation of hormone secretion, the use of a specific antagonist, such as naloxone, devoid of side effects, seems preferable.

Naloxone administration in humans has been shown to cause a pressor effect in patients affected by shock of different aetiology (Peters et al. 1981; Dirksen et al. 1980; Lenz et al. 1981) and sometimes to provoke hypertensive crises when administered to reverse anaesthesia (Estilo & Cottrell 1981; Tanaka 1974) but in normal volunteers and in basal conditions it has been shown to be unable to modify BP consistently (Zilm 1980; Rubin et al. 1981; Estilo & Cottrell 1982) when injected in doses as high as 20 mg. Only very high doses (in the mg/kg range) have been shown to produce dose-dependent increases in systolic BP and respiratory rate, in normal adults (Cohen et al. 1982). Therefore our results on BP and HR are in agreement with those in the literature. Moreover these results are in agreement with the inability of naloxone to modify NE peripheral levels (Table 3).

The inability of naloxone, when administered in low doses (0.2, 0.4 mg), to modify CA plasma levels in normal volunteers has already been demonstrated by Estilo & Cottrell (1982) and is in agreement with our data.

To our knowledge no data exist on the effects of higher doses of naloxone on E plasma levels in adult man.

In our study 10 mg naloxone was able to increase significantly E peripheral concentrations (Fig. 1). The increase was slight and unable to modify BP and HR but significant (P < 0.01) and was observed in each subject studied.

It is worth mentioning that in a 31 year old patient affected by hypertensive crises due to bilateral phaeochromocytoma (surgically removed) IV administration was able, on two separate occasions, to induce an increase in BP and CA plasma levels, while 0.4 mg naloxone was as ineffective as placebo administration (Mannelli et al. 1983).

These results, taken together, seem to support the hypothesis of an opioid inhibitory role for CA release from the adrenal medulla in man and are in agreement with other experimental data obtained in vitro (Kumakura et al. 1980) and in vivo (Tabor-sky et al. 1982).

The finding that only high doses of naloxone were able to induce a rise in E plasma levels seems to suggest that receptors other than µ are involved in the modulation of adrenal medullary secretion.

From our data it is not possible to determine whether the action of naloxone is centrally or peripherally mediated.

Further studies are necessary to evaluate whether naloxone may have an immediate effect on CA plasma levels following injection. Moreover, as the increment observed in E plasma levels, although significant, is slight, further studies, possibly in conditions other than basal, are necessary better to evaluate the physiological significance of the modulation opiates seem to exert on the human adrenal medulla.

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<table>
<thead>
<tr>
<th>Time</th>
<th>Placebo</th>
<th>0.4 mg</th>
<th>4.8 mg</th>
<th>10 mg</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>100 ± 20</td>
<td>104 ± 15</td>
<td>100 ± 18</td>
<td>99 ± 9</td>
</tr>
<tr>
<td>+ 15</td>
<td>106 ± 16</td>
<td>103 ± 21</td>
<td>159 ± 35</td>
<td>186 ± 23* **</td>
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<tr>
<td>+ 30</td>
<td>112 ± 20</td>
<td>124 ± 23</td>
<td>132 ± 18</td>
<td>152 ± 29</td>
</tr>
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</table>

Significance: vs time 0: * P < 0.01; vs placebo ** P < 0.02.

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<table>
<thead>
<tr>
<th>Time</th>
<th>Placebo</th>
<th>4.8 mg</th>
<th>10 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>768 ± 66</td>
<td>702 ± 107</td>
<td>803 ± 60</td>
</tr>
<tr>
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<td>813 ± 87</td>
<td>674 ± 60</td>
<td>869 ± 44</td>
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<tr>
<td>+ 30</td>
<td>860 ± 87</td>
<td>650 ± 50</td>
<td>860 ± 64</td>
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</tbody>
</table>

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Table 3.
Mean (± SEM) epinephrine plasma levels (pmol/l) in 8 subjects after placebo or different doses of naloxone.

Table 4.
Mean (± SEM) norepinephrine plasma levels (pmol/l) in 8 subjects after placebo or different doses of naloxone.
Acknowledgments

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References


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