The effects of chronic consumption of heroin on basal and vagal electrical-stimulated gastric acid and pepsin secretion in rat

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ABSTRACT

Objectives: Addiction to opium and heroin is not only an important social and individual problem in the world but it also affects the human physiology and multiple systems. The aim of this study is to determine the effects of chronic heroin consumption on basal and vagus electrical-stimulated total gastric acid and pepsin secretion in rats.

Methods: The study was carried out in the Department of Physiology, Kerman University of Medical Sciences, Iran from August 2002 to June 2003. Both male and female rats weighing 200-250g were used. Rats received daily doses of heroin intraperitoneally starting from 0.2 mg/kg to 0.1mg/kg/day up to the maintenance level of 0.7mg/kg and continued until day 12. After anesthesia, tracheotomy and laparotomy, gastric effluents were collected by washout technique with a 15 minutes interval. The total titrable acid was measured by manual titrator, and the total pepsin content was measured by Anson's method. Vagal electrical stimulation was used to stimulate the secretion of acid and pepsin.

Results: Heroin results in a significant decrease in total basal acid and pepsin secretions (4.10 ± 0.18mmol/15 minutes versus 2.40 ± 0.16mmol/15 minutes for acid, $p<0.01$, and 3.63 ± 0.18 mg/15 minutes versus 3.11± 0.18 mg/15 minutes for pepsin, $p<0.05$). But, it does not produce any significant changes in acid and pepsin secretions in vagotomized condition. Heroin also causes a significant decrease in vagal-electrically stimulated acid and pepsin secretions (14.70 ± 0.54 mmol/15 minutes versus 4.30 ± 0.21mmol/15 minutes for acid, $p<0.01$, and 3.92 ± 0.16 mg/15 minutes versus 3.37± 0.16 mg/15 minutes for pepsin, $p<0.05$).

Conclusion: Heroin consumption decreases the total gastric basal and vagus stimulation of acid and pepsin secretion, but not in vagotomized condition. Heroin may decrease acid secretion by inhibiting vagal release of acetylcholine within the gastric wall. Other probable mechanisms include: presynaptic inhibition of acetylcholine release or depressing the vagal center, inhibition of pentagastrin induced acid secretion, inhibitory effects via central mechanisms, probably mediated by the opiate receptors. Further studies are needed to recognize the actual mechanism.

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Addiction to narcotic substances such as heroin is not only an important social problem through the world but also has adverse effects on body organs and their physiology. The importance of this fact has lead to special attention of World Health Organization (WHO) to the prevention of addiction, and to treat the physical and psychological disorders of abuser. Heroin (3,6-diacetylmorphine) is an opioid with high addictive ability and has of the highest rates of consumption among narcotic drugs.[1] It is recognized that heroin produces more pronounce pharmacological effects and is more

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addictive than morphine.[1] It is consumed by various way but mostly in the form of injection. Pure heroin may be smoked.[1] Previous studies have shown that 80% of heroin abusers are under 26-year-old.[1] Heroin exerts its effect as an agonist for mμ, kappa and delta receptors in the central nervous system. The receptors of mμ[2] are responsible for hypomotility in digestive system.[2,3] The distribution of opioids and their receptors in body organs such as digestive and central nervous systems can affect the motility and secretory activities of digestive system. It has been observed that opioids can affect gastric acid secretion via 3 mechanisms:[4] (1) Central effect on brain, (2) indirect peripheral effect and (3) direct effect on parietal cells. Heroin is a strong opioid with a lot of harmful effects in the body. Nabavizadeh Rafsanjani et al[5] have investigated the effects of acute heroin consumption on acid and pepsin secretion.[5] The present study shows the effect of chronic consumption of pure heroin on basal and vagal-electrically stimulated acid and pepsin secretion.

Methods. In this study, 20 N-mari rats of both sexes with mean weight of 200-250 grams were used. Animals were kept in the animal house of Kerman Medical University, Kerman, Iran at a temperature of 25±2 centigrade with 12 hours dark/12 hours light cycle. They were fed with standard food and were divided into 2 groups: case group (N=10) and control group (N=10). Control group had an access to normal food and water. Animals in the case group were addicted to heroin by gradual dose increase method by pure heroin. The procedure was started with 0.2 mg/kg (body weight) on the first day and the dosage was increased by 0.1 mg/kg daily until 0.7 mg/kg body weight was reached. This dose was continued until the 12th day.[6] At the end of the 12th day, some rats were selected randomly and 2 mg/kg naloxan was injected subcutaneously each rat and was put in a glass cabinet and considered for appearance of withdrawal symptoms for 20 minutes. Withdrawal symptoms were jumping, head shakes, diarrhea, irritability, paw tremor, ptosis, writhing, chattering teeth and straub tail.[7] Each animal was considered as an addict if 4 or more of the mentioned symptoms appeared.[7]

Before the experiments, animals were deprived of food for 24 hours but had a free access to water.[8] In control group after weighing animals, they were anesthetized by intraperitoneal injection of sodium thiopental (nesdonal 50 mg/kg body weight),[9] while in the case group, to prevent death due to respiratory depression, 40 mg/kg body weight was applied. After anesthesia tracheostomy was carried out and esophagus was tied in neck region to prevent reflux aspiration.[8] Then, the vagus nerve in both sides was carefully isolated from carotid artery for approximately 1 cm and anchored by a string and finally covered with cotton soaked in normal saline.[10] Following this stage, laparotomy was carried out by an incision of approximately 2 cm long in mid-line and a silicon tube (external diameter = 2.5 mm) was entered into the stomach via duodenum. For emptying the probable remaining contents of stomach, it was washed several times with 1-1.5 ml normal saline (T=37ºC, pH=7).[11] Then the vagus nerve was ligated tightly and the nerve was cut in central part. For measuring basal acid secretion, 1 ml ringer solution was entered into the stomach and after 15 minutes another 1 ml was added and the stomach content was emptied and 1 ml of that was titrated. This procedure was repeated twice. Acid measurement was carried out by titrator instrument (W. Germany, DIN) and by using 0.01 N sodium hydroxide. In order to measure vagal-stimulated acid secretion. The peripheral part of right vagus nerve was stimulated by a stimulator (V=15V, F=4 Hz, W=1 msec) for 15 minutes and then acid secretion was measured every 15 minutes until reaching the basal state again. The voltage and frequency of stimulation were chosen in a way that no cardio-vascular problem would arise.[11] Pepsin secretion in all stages of basal, vagotomy, vagal-stimulation and returning to basal state was measured by Anson's method[12] and with hemoglobin as pepsin enzyme substrate.[13] Acid and pepsin secretion were recorded as mean±SE. Data analysis were carried out by using Student's t-test and $p<0.05$ was considered statistically significant.

Results. The results showed that acid secretion at minutes of 15 and 30 were 4.10±0.18 mmol/15 minutes and 2.3±0.15 mmol/15 minutes in control group and 2.4±0.16 mmol/15 minutes and 1.2 ±0.13 mmol/15 minutes in case group. There was significant difference between the 2 groups in this regard, $p<0.01$, (**Figure 1**). There was no significant difference in acid secretion at minutes of 45 and 60 of vagotomy state between case and control groups (**Figure 1**). After 15 minutes vagal stimulation, acid secretion was 4.30±0.21 mmol/15 minutes in case group and 14.7±0.54 mmol/15 minutes in control group, this decrease in case group was significant ($p<0.01$) (**Figure 1**). Acid secretion at minutes 90 and 105 of returning to vagotomy state was 9.6±0.65 and 4.10±0.64 mmol/15 minutes in control group ($p<0.01$) and 1.8±0.20 and 1±0.20 mmol/15 minutes in case group, $p<0.05$ (**Figure 1**). Basal pepsin secretion at the minutes of 15 and 30 were 3.63±0.18 mg/15 minutes and 3.32±0.16mg/15 minutes in control group and they were 3.11±0.18 mg/15 minutes and 3.09±0.12mg/15 minutes in the case group at the same minutes. There was a significant decrease in basal pepsin secretion in case

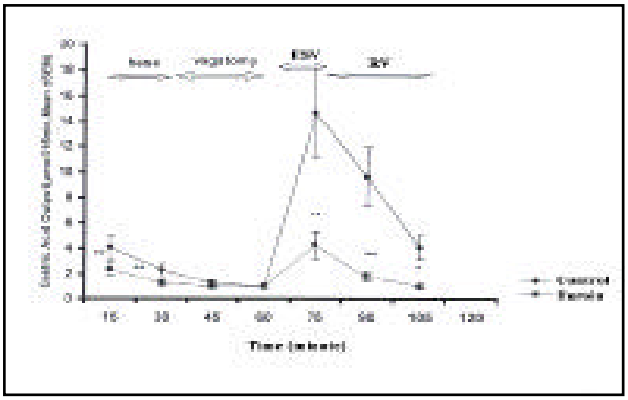

Figure 1 - Comparison of the acid secretion between the control and case group in rats. ESV - end-systolic volume, RV - residual volume.

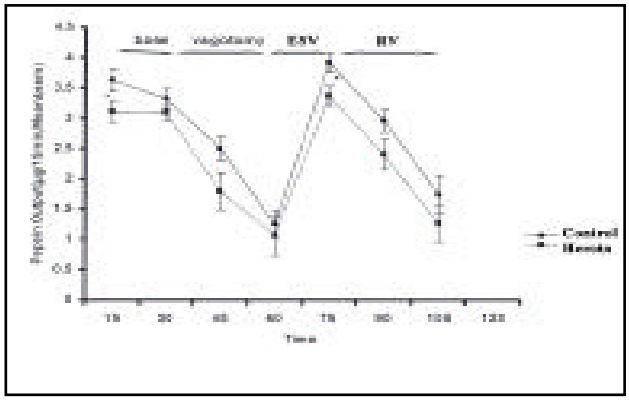

Figure 2 - Comparison of the pepsin secretion between the control and case group in rats. ESV - end-systolic volume, RV - residual volume.

group comparing with control group, $p<0.05$, (**Figure 2**). There was a decreased pepsin secretion in case group at minutes 45 and 65 of vagotomy, but it was not significant (**Figure 2**). After vagal stimulation, pepsin secretion was 3.92±0.16mg/15 minutes in control group and 3.37±0.16mg/15 minutes in case group, $p<0.05$, (**Figure 2**). There was no significant difference between the 2 groups in pepsin secretion during all stages of returning to vagotomy state (**Figure 2**).

Discussion. The results of this study showed that chronic heroin usage decreases basal and vagal electrical-stimulated acid and pepsin secretions (**Figures 1 & 2**). In the previous studies on the effects of morphine on acid secretion (μ agonist) in rat, a decrease or no difference in the rate of acid secretion has been reported.[14-16] In another study, acute consumption of heroin has lead to an increase in the basal acid secretion.[5] It has been reported that vagus electrical stimulation increase acid and pepsin secretion by acetylcholine release and via various mechanisms:[9,17,18] (i) Acetylcholine exerts its effect directly on its receptor on parietal cells and increases gastric secretion. (ii) Acetylcholine increases gastric secretion in directly via increasing histamine release. (iii) Acetylcholine inhibits the suppressive effects of somatostatin on gastric secretion. (iv) Acetylcholine increases gastric secretion via gastrin release.

The acetylcholine receptors on parietal cells are of M3 type. With vagal stimulation, acetylcholine is released from cholinergic post ganglionic neurons located near the parietal cells. After binding to its receptor, and in the presence of cytoplasmic calcium, acetylcholine activates phospholipase C and therefore leads to an increase in the activity of potassium-hydrogen pump in parietal cells and consequently, in acid secretion.[19] In the present study, vagal electrically stimulated gastric acid and pepsin secretion in both control and case group showed a significant increase in comparison to basal secretions. Basal and vagal stimulated acid secretion of case group showed a significant decrease compared with that of control group. The maximum secretory response was observed in 2-4 HZ frequency. Vagal stimulation in higher frequencies decreases acid secretion may be due to a decrease in arterial pressure and blood flow in the stomach mucous. It seems that voltage, frequency and duration of stimulation are the major determinating factors in secretary response intensity. The difference between the amount of acid secretion in the present study and other study can be due to the same fact. In this study, the increase in acid and pepsin secretion continued for some time after stopping the vagal stimulation. This can be due to the presence of gastrin in blood[18] or histamine.[20] Returning to basal state was different in the 2 groups that can be related to the heroin effect. It has been documented that opioid receptors are present in both, solitarius nucleus (NST) in brain stem and vagus trunks. Also, NST in brain stem is one of the involved nuclei in regulating acid release in stomach.[21] Therefore, acting through this nucleus may be one of the mechanisms of heroin effect on acid secretion. It has been proved that morphine decreases acid secretion via central inhibitory mechanisms in rat. It has also been reported that opioids suppress acetylcholine release from cholinergic nerves in stomach and decrease vagal and pentagastrin-stimulated acid secretion.[22] Patton[23] have reported that opioids decrease acetylcholine release in all tissues. In addition to pre-synaptic suppressive effect of opioids, acetylcholine release suppression via submocusal network in rat's colon has been also reported. In the present study, chronic consumption of heroin led to a significant decrease in basal and vagal electrically stimulated acid and pepsin secretion probably via the mentioned mechanisms. As long as

there is no other study carried out regarding the effect of other narcotic drugs on pepsin secretion, authors could not compare their findings on pepsin secretion with others. Heroin consumption decreases basal and vagal stimulated acid and pepsin secretion, but not in vagotomized condition. Heroin may decrease acid secretion by inhibiting vagal release of acetylcholine within the gastric wall. Other probable mechanisms include: presynaptic inhibition of acetylcholine release or depression of the vagal center, inhibition of pentagastrin induced acid secretion, inhibitory actions via central mechanisms probably mediated by the opiate receptors. Further studies should be carried out to find the main or actual mechanism. Our previous study[5] has shown that acute heroin consumption increases gastric acid and pepsin secretion, but in this study chronic consumption followed by decrease gastric secretion.

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