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Immunoreactive "calcitonin-like" material in heroin addicts: varying reactivity with different antibodies

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Summary. High levels of immunoreactive calcitonin (iCT) in the blood of heroin addicts were previously reported. As it is well known that multiple forms of calcitonin exist in the blood and in tissues, the purpose of the present study was to investigate the immunological nature of the CT-like immunoreactive material found in the blood of these subjects. We investigated 25 addicts, who had been using heroin for more than one year and were hospitalized for a 2 week detoxication program. Blood samples were drawn at the start of the program (when the subjects were still on heroin) and after 5 and 12 days of abstinence from heroin. Twenty-five healthy subjects served as controls. We used 2 commercial RIA kits, calibrated against the same reference material (WHO 70-234), but employing different antisera. One antiserum substantially confirmed the previous findings of increased levels of calcitonin during heroin use, but the other seemed to exclude any change in the hormone concentrations. This suggests that the "calcitonin like" material found in heroin addicts contains some epitopes similar to those found in the calcitonin standard which are detected by the first antiserum. However it lacks other epitopes which are also present in calcitonin standard and which are recognized by the second antiserum. Therefore, this substance seems to be different from the standard human calcitonin 1-32. A possible involvement of a calcitonin analogue (precursor or metabolite) in the biochemical changes occurring during chronic opiate use is suggested.

Key words: Heroin addiction – Calcitonin – Immunoheterogeneity

Zusammenfassung. Über hohe Spiegel von immunoreaktivem Calcitonin (iCT) im Blut von Heroinsüchtigen wurde in vorhergehenden Studien berichtet. Da bekannt ist, daß zahlreiche Formen von Calcitonin im Blut und in den Geweben existieren, war es das Ziel der vorliegenden Studie, die immunologische Natur des calcitoninähnlichen immunreaktiven Materials zu untersuchen, welches im Blut dieser Personengruppe gefunden wird. Wir untersuchten 25 Süchtige, welche seit mehr als 1 Jahr Heroin nahmen und während eines Entgiftungsprogramms mehr als zwei Wochen hospitalisiert waren. Blutproben wurden zu Beginn des Programms genommen (als die Probanden noch heroinbelastet waren) und nach 5 bzw. 12 Tagen der Abstinenz. 25 gesunde Versuchspersonen dienten als Kontrollgruppe. Wir benutzten zwei kommerzielle RIA Kits, welche gegen dasselbe Referenzmaterial (WHO 70-234) calibriert waren, jedoch unterschiedliche Antiseren beinhalten. Ein Antiserum stimmte substanziell mit vorhergehenden Beobachtungen überein, nach denen die Calcitonin-Spiegel während des Gebrauchs von Heroin erhöht sind, das andere jedoch schien jede Veränderung der Hormon-Konzentrationen auszuschließen. Dieses Ergebnis deutet darauf hin, daß das "calcitoninähnliche Material", welches bei Heroinsüchtigen gefunden wird, einige Epitope enthält, welche analog dem Calcitonin-Standard sind und durch das erste Antiserum erfaßt werden. Das "calcitoninähnliche Material" scheint jedoch andere Epitope nicht aufzuweisen, welche im Calcitonin-Standard vorhanden sind und welche durch das zweite Antiserum detektiert werden. Daher scheint diese Substanz sich vom Standard-Calcitonin 1-32 zu unterscheiden. Es wird diskutiert, daß möglicherweise ein Calcitonin-Analogon (Precursor oder Metabolit) in die biochemischen Veränderungen während des chronischen Opiatmißbrauchs involviert ist.

Schlüsselwörter: Heroinsucht – Calcitonin – Immunologische Hetereogenität

Introduction

Calcitonin (CT) is a 32 amino acid peptide secreted by the parafollicular "C" cells of the thyroid. Its hypocalcemic activity was first described in 1962 [1], but its physiological role in man and mammals has not yet been fully

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clarified. In general, it is considered to be a hormone protecting the skeleton during periods of physiological stress, such as growth, pregnancy and lactation, by reducing the calcium loss [2].

On the other hand, evidence of an activity of CT as a neuropeptide involved in the modulation of pain has been increasing, after the first paper from Pecile and coworkers appeared in 1975 [3]. In fact, CT injected inside the blood-brain barrier, has shown a potent analgesic activity in different animal species [3–6] and in man [7]. Even the peripheral injection of CT has been found to enhance the pain threshold [5]. Most reports suggest that centrally induced CT analgesia is independent of opioid mechanisms [8], although there is not complete agreement on this point [9]. In addition, immunoreactive calcitonin (iCT) and specific binding sites in the central nervous system and in the pituitary gland have been reported by several authors [10–12]. These findings have prompted us to study the behaviour of CT in chronic opiate consumers such as heroin addicts. As early as 1984, we found that circulating levels of iCT in heroin addicts were significantly higher than in controls [13]. Further studies by different groups confirmed these findings and demonstrated that abstinence from the drug caused a decrease in the CT concentrations [14, 15].

As the presence of multiple forms of CT in blood and in tissues has already been reported [16], the purpose of the present study was to investigate the antigenic nature of the CT-like immunoreactive material found in the blood of heroin addicts.

Materials and methods

We investigated 25 subjects (20 males and 5 females, aged 18–34 years), who had been using heroin for more than one year and were hospitalized for a 2 week detoxication program, during which only tapered doses (from 250 to 0 mg/day in 2 weeks) of a mild antidepressant, antiserotoninergic drug (Trazodone, Angelini, Rome) were administered. In our experience, this drug shows no measurable effect on CT levels (personal, unpublished data available on request).

After an overnight fast, 3 blood samples were drawn (at 8 a.m.) at the start of the program (when the subjects were still on heroin) and after 5 and 12 days of treatment, with complete abstinence from heroin. Sodium EDTA (2 mg/mL) and 1000 KIU/mL of aprotinin (Trasylol, Bayer, FRG), a serum protease inhibitor, were added to all the blood samples; plasma was separated within 30 min and stored at −20°C until assayed.

Screening for urinary opiates was carried out using an automated enzyme immunoassay (EMIT, Syva, Palo Alto, CA) at admission and then every day during the program. The test was positive on admission and negative during hospitalization in all the subjects.

No subject showed clinical symptoms or biochemical signs of impaired liver or kidney function; serum transaminases, bilirubin, calcium, phosphorus, creatinine, alkaline phosphatase and C-PTH were within the reference values. Twenty-five healthy subjects (20 males and 5 females, aged 18–34 years), without any clinical symptoms or biochemical signs of impaired liver or kidney function and without evidence of opioid or psychoactive drugs intake (confirmed by an EMIT urine screening) served as controls.

Methods. iCT was measured by 2 different assays, using standard curves of human CT 1-32, calibrated with the same reference material (WHO 70-234):

1) RIA *Kit A,* marketed by DPC (Los Angeles, CA, USA), employs a goat antiserum, a double antibody-PEG separation and requires an initial incubation of 44 h and an incubation of 18 h with the tracer.

It is stated that no cross-reactions occurs with CGRP, salmon and eel CT, PTH 1-84, PTH 1-34, PTH 53-84 and PTH 44-68. The limit of detection (i.e. the amount of CT which lowers the binding capacity of 5% as compared to the 0 standard) was 5 pg/mL.

2) RIA *Kit B,* marketed by Nichols Institute (Los Angeles, CA, USA), employs a goat antiserum, a double antibody-PEG separation with a similar incubation procedure. No cross-reactions occur with CGRP, salmon CT, PTH 1-84 and PTH 1-34. The limit of detection was 3 pg/mL.

The results were analyzed using paired and unpaired "Student's *t*-test".

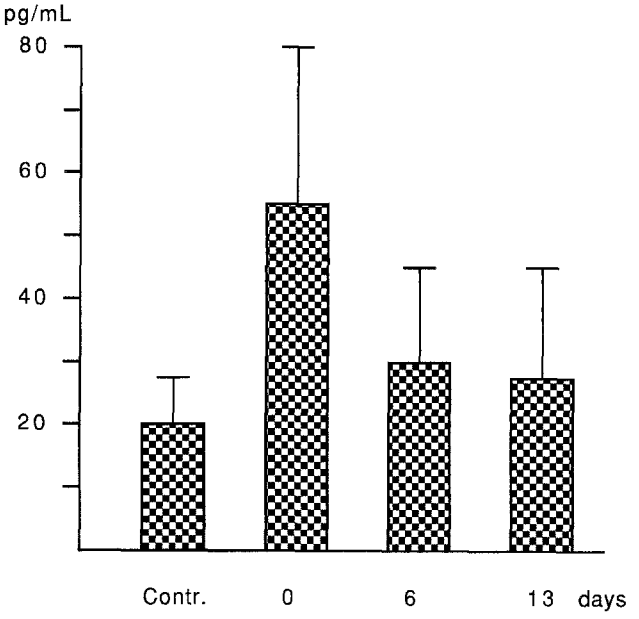

Fig. 1. iCT (mean ± SD) measured with antiserum A in controls and in heroin addicts at days 1, 6 and 13 of the detoxication program

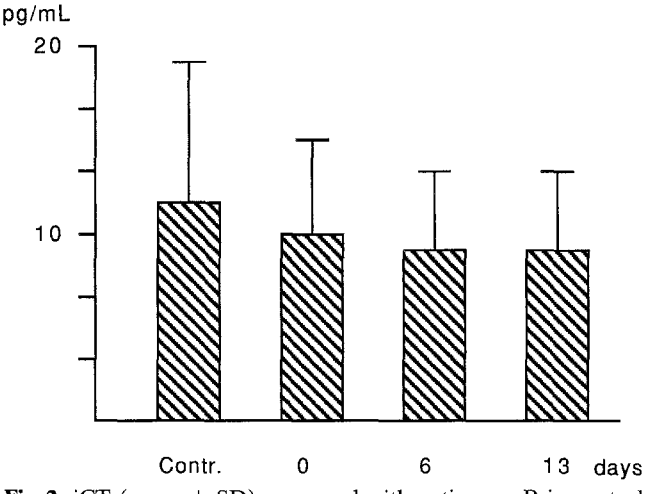

Fig. 2. iCT (mean ± SD) measured with antiserum B in controls and in heroin addicts at days 1, 6 and 13 of the detoxication program

Table 1. Mean CT levels (pg/mL) and in plasma of heroin addicts and controls measured with antisera A and B

	Day 0	Day 6	Day 13	Controls
CT, antiserum A	55.92	30.71	28.52	19.40
S.D.	22.41	16.88	26.62	8.17
CT, antiserum B	9.72	8.56	9.08	10.91
S.D.	4.54	4.95	4.69	7.86
Statistical evaluation				
CT, antiserum A:	day 0 > controls: $P < 0.0005$			
	day 0 > day 6: $P < 0.0005$			
	day 0 > day 13: $P < 0.0005$			
	day 6 > controls: $P = 0.018$			
	day 13 *vs.* controls: not significant			
	day 6 *vs.* day 13: not significant			
CT, antiserum B:	day 0 *vs.* controls: not significant			
	day 0 *vs.* day 6: not significant			
	day 0 *vs.* day 13: not significant			
	day 6 *vs.* controls: not significant			
	day 13 *bs.* controls: not significant			
	day 6 *vs.* day 13: not significant			

Results

The results are shown in Fig. 1 and 2 and in Table 1.

Antiserum A: the mean concentration of iCT found in addicts still on heroin was significantly higher than in the controls ($P < 0.0005$) and fell significantly during the abstinence from the drug ($P < 0.0005$). The iCT concentrations in the control group and in the heroin addicts on the 6th and 13th day of the detoxication program were not statistically different.

Antiserum B: the mean concentration of iCT at the start of the program was not different from the controls and did not change during the abstinence from heroin. In addition, the average concentration of iCT in the controls was significantly lower than the concentrations measured with the DPC assay in the same group ($P < 0.0005$).

Discussion

The increase of iCT in the blood of heroin addicts and the decrease during abstinence from the drug has already been reported by different groups [13–15, 17, 18]. The behaviour of iCT was confirmed using an extraction and purification procedure with ODS-silica cartridges [17]. This is a similar method to that which, according to Body and Heath [19], allows the separation of monomeric CT. These findings are substantially in agreement with the present results obtained with antiserum A.

On the other hand, the results from antiserum B seem to exclude any change of iCT levels related to heroin use. In fact, plasma concentrations of the hormone in the heroin addicts were similar to the controls and did not change at all during the detoxication program.

It is difficult to explain this discrepancy, taking into account that both RIA's for CT were calibrated against the same standard (WHO 70-234). It is clear that, although both antisera bind standard CT, the DPC antiserum (A) recognizes as CT a compound that is not recognized by the Nichols antiserum (B). This suggests that the "CT-like" material found in heroin addicts contains some epitopes similar to the CT standard, detected by antiserum A, but lacks other epitopes, present also in the CT standard, which are recognized by antiserum B. In short, this material seems to be different from human CT 1-32 (the used standard).

Since CT is cleaved from pre-pro-calcitonin, a much larger precursor polypeptide of 141 amino acids, several precursors of different molecular weight and several degradation products, with conceivably varying immunological characteristics, may co-exist in blood with monomeric CT. One or more of these CT-like peptides could increase in the blood of heroin users, suggesting a possible role of CT in the biochemical changes occurring during chronic opiate use. A potential of this compound(s) as a "marker" of heroin intake could also be hypothesized.

However, we think that direct immunological methods can give us no more than a glimpse of the problem. A deeper understanding of CT changes in heroin addicts could be assured only by a HPLC purification prior to RIA, in order to compare the chromatographic pattern of the iCT of heroin addict, the iCT of normal subjects and the standard CT 1-32.

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