Journal of Psychiatric Research 44 (2010) 1148–1153

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/psychires

CCK-4: Psychophysiological conditioning elicits features of spontaneous panic attacks

Kim Hinkelmann [a,*], Alexander Yassouridis [b], Reinhard Mass [c], Henrike Tenge [a], Michael Kellner [a], Holger Jahn [a], Klaus Wiedemann [a], Karsten Wolf [c]

[a] Department of Psychiatry and Psychotherapy, University of Hamburg, Martinistr. 52, 20246 Hamburg, Germany
[b] RG Statistics, Max-Planck-Institute of Psychiatry, Kraepelinstr. 2-10, 80804 Munich, Germany
[c] Center of Mental Health Marienheide, Leppestr. 65-67, 51709 Marienheide, Germany

ARTICLE INFO

Article history:
Received 22 October 2009
Received in revised form
1 April 2010
Accepted 6 April 2010

Keywords:
Panic anxiety
CCK-4
Emotional facial expression
Facial EMG
HPA-axis
Conditioning
Activation density

ABSTRACT

Introduction: Cholecystokinin-tetrapeptide (CCK-4) is an established model to generate subjective panic anxiety. CCK-4 injection also results in consistent and dose-dependent rise of stress hormones. Effects other than upon subjective panic and stress hormone activity have barely been examined. The purpose of the study was to investigate CCK-4 effects on emotional facial expression and especially on fear relevant facial muscles establishing therewith a more objective method to measure subjective panic anxiety.
Methods: 20 healthy male subjects were randomly and double-blindedly assigned in two groups (dose groups), each of which was investigated three times once with placebo and twice with 25 μg or 50 μg CCK-4 respectively. Subjects of each group were randomly assigned in two different balanced orders of investigations: CCK-CCK-Placebo vs. Placebo-CCK-CCK. Facial muscle and hypothalamo-pituitary-adrenocortical (HPA)-axis activity were recorded.
Results: CCK-4 led dose-dependently to an increase of panic anxiety, an activation of fear relevant facial muscles and a rise of stress hormones. Whereas placebo administration before CCK-4 revealed no significant panic and stress response, during placebo following CCK-4 stimulations a psychophysiological conditioning effect could be observed without rise in HPA-axis activity.
Discussion: Our findings indicate the possibility to measure different intensities of panic anxiety and conditioning effects with a facial EMG method. Dissociation of HPA-activity and fear relevant facial muscle activity is in accordance with former results about spontaneous panic attacks.

1. Introduction

Intravenous injection of cholecystokinin-tetrapeptide (CCK-4) is an established model to experimentally induce panic-like anxiety not only in patients, but also in healthy volunteers (Bradwejn et al., 1995; Kellner et al., 2002; Wiedemann et al., 2001). CCK-4 exerts its effects via cholecystokinine B-type receptors in the CNS. Panicogenic effects are consistent, reproducible, related to the dose administered (Bradwejn et al., 1992) and closely resemble spontaneously occurring panic attacks (Bradwejn et al., 1990). fMRI studies showed that CCK-4 administration leads to brain activation in fear relevant neuronal circuits (Eser et al., 2009b; Schunck et al., 2006). In addition to its panicogenic effects, CCK-4 activates hypothalamo-pituitary-adrenocortical (HPA) hormone releases via corticotropin-releasing hormone (CRH) in patients as well as in healthy subjects (Eser et al., 2007; Wiedemann et al., 2001).

Although the CCK-4 model has been evaluated extensively in the last years, the assessment of induced panic anxiety other than subjective panic response has raised difficulties, since cardiovascular and neuroendocrine response do not reflect a valid read out (Eser et al., 2007).

However, CCK-4 effects other than subjective panic experience and neuroendocrine responses have barely been studied: Gunnarsson et al. investigated the effects of CCK-4 on brain stem auditory evoked potentials in healthy subjects. Their results support that CCK-4 administration may be a useful challenge paradigm for investigating CCK's modulatory role on brain stem mechanisms mediating anxiety and panic in humans (Gunnarsson et al., 2003). Eser et al. (2009a) investigated the impact of loudness dependency of auditory evoked potentials on the panic response to CCK-4, which did not differ between panickers and non-panickers.

* Corresponding author. University Hospital Hamburg-Eppendorf, Department of Psychiatry, Martinistr. 52, 20246 Hamburg, Germany. Tel.: +49 40 42803 4222; fax: +49 40 42803 4207.
E-mail address: hinkelma@uke.uni-hamburg.de (K. Hinkelmann).

0022-3956/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jpsychires.2010.04.004

The emotional facial expression by fear related muscle activity could provide an objective indicator for individuals' experience of panic anxiety. To our knowledge CCK-4 effects on fear related mimic muscles have not been investigated so far. That anxiety is represented through activity by M. frontalis and M. risorius and secondarily by the platysma was established by Ekman and Friesen (2002, 2003).

This facial pattern has been found to be expressed during high-anxious segments of self-reported past events (Harrigan and O'Connel, 1996) and is related to enhanced sensory acquisition (Susskind et al., 2008).

Darwin (1872) declared three muscles as specific for anxiety: M. frontalis, M. corrugator and platysma. Furthermore he described different anxiety expressions depending on panic anxiety intensity, but without giving the detailed changes of facial expression. Also a more recent study suggests a relationship between anxiety intensity and facial expression, especially in the perioral area (Nakamura, 2002).

Aim of our present study was to explore the emotion driven muscle pattern of panic anxiety after CCK-4 by using a highly sensitive facial EMG method. This EMG method allows an improved analysis and discrimination of diminutive pre-visible facial muscle activity by measuring independently up to nine facial muscles. The validity of facial EMG in general has been proved by several investigations (Dimberg et al., 2000; Wolf et al., 2005).

Our hypotheses were, that a) administration of CCK-4 induces activity increments in fear relevant but not in other facial muscles, b) the *pattern* of facial muscle activity is dependent from CCK-4 dosage and c) repeated CCK-4 stimuli lead to psychotropic conditioning. Fear relevant muscles were defined according to Ekman (M. frontalis, M. risorius and platysma) and were compared to other facial muscles (orbicularis oculi, zygomaticus).

2. Method

2.1. Subjects

Twenty healthy male subjects (mean age 24.7 years; range 19–31 years) were studied in a prospective, randomized, double-blind, placebo-controlled and balanced design. Subjects were free of former and present DSM-IV axis I and axis II disorders according to MINI and psychiatric interviews, had no physical illness according to medical examination and standard blood lab, including negative urinary drug screen and had been free of any medication for at least 3 months. Subjects were recruited by means of advertisement. The study was approved by the local ethics committee of Hamburg and written informed consent was obtained from each participant prior to the study.

2.2. Groups and subgroups

Each subject was studied three times. Subjects were randomly divided into two groups: The first group ($n = 10$) received two times a dosage of 25 μg CCK-4 dissolved in 10 ml of isotonic sodium chloride vs. placebo (=10 ml of isotonic sodium chloride), the second group ($n = 10$) two times a dosage of 50 μg CCK-4 vs. placebo to investigate dose-dependent muscle reactions. To control for order effects and for analysis of conditioning and sensitation effects, subjects of each group were again randomly assigned in two different subgroups receiving the order CCK-CCK-Placebo or Placebo-CCK-CCK, correspondingly. The compounds to be administered were prepared by a co-worker not involved in the conduction of the study and blind to the order.

2.3. Procedure

All subjects were tested under video observation in an electrically shielded, soundproof private room from 09:00 to 13:00 on three separate days with a minimum interval of one day. Subjects were allowed to adapt to the surrounding for 2 h. An intravenous cannula was inserted into a left forearm vein at 09:00. The cannulae were kept patent using isotonic sodium chloride solution at a flow rate 50 ml/h. At 11:00 each subject received as bolus injection within 20 s (Clinalfa AG, Läufelingen, Switzerland), 25 μg/50 μg CCK-4 dissolved in 10 ml of isotonic sodium chloride or placebo (=10 ml of isotonic sodium chloride). Only for the double blind injection of CCK-4 vs. placebo an investigator entered the room. During the CCK-4 vs. placebo challenge participants were exposed to a gray-colored picture to implement a psychological control.

Blood samples were drawn at 10:30, 10:50, 11:05, 11:10, 11:15, 11:30 and 12:00 to measure hormonal concentrations. These samples were placed on ice, plasma was immediately separated, and specimens were stored at −80 °C until analyses. Blood pressure was registered at blood sampling times with an automatic device.

2.4. EMG measures

Bipolar EMG recordings (AEF™, Karlsruhe/Hamburg, Germany) were taken from nine muscles, namely from the M. frontalis medialis, M. corrugator supercilii, M. orbicularis oculi, M. levator labii superioris, M. zygomaticus, M. risorius, platysma, M. depressor anguli oris and M. mentalis region on the left side of the face, using Hellige miniature surface Ag/AgCl electrodes (inside diameter 0.6 cm) filled with Med-Tek/Synapse conductive electrode cream. Electrodes were placed according to the recommendations of Fridlund and Cacioppo (1986) with the exception of the electrodes over the M. risorius and the platysma, placed according to anatomical knowledge. The inter-electrode distances were 12 mm. Skin was prepared by abrasion with 70% clinical alcohol first and Hellige Epicont abrasive skin preparation cream secondly. The electrodes were connected to Becker MEDITEC™ Amplifiers. The raw EMG signal was analyzed automatically by a two channel contour-following integrator (Varioport™, Becker MEDITEC™, Karlsruhe, Germany). Frequency range (-3 dB) of 90–500 Hz with a time constant of 0.0018 s. Amplification factor 5000 (+/−2%), CMRR (Common Mode Rejection Ratio) 77 dB at 50 Hz, time constant of integrator 0.1 s, Range +/−250 μV, Resolution of AD-Converter 12 Bit (=4096 steps), resolution of signal 0.122 μV per step. The input impedance is theoretically 1 GOhm, due to our cable capacities of about 500 MOhm at 50 Hz. The sampling rate was 32 Hz. The output signals were recorded and stored in computer files (Variograph™ software on a Macintosh Powerbook, Becker MEDITEC™, Karlsruhe, Germany) for off-line analysis. With the help of video recordings the complete EMG data were screened for artifacts like eye blinking. Artifacts were interpolated with the Variograph™ software. The injection of 25 μg and 50 μg CCK-4 vs. placebo was done within 20 s from 11:00:00 to 11:00:20 h. The earliest CCK-4 effect was expected at 11:01:20 h (60 s after injection). For facial EMG analysis baseline was defined as 11:01:17–11:01:20 (3 s before earliest treatment effect), trial phase was defined as 11:01:20–11:01:40 (between 60 and 80 s after injection).

2.5. Assessment of panic anxiety

CCK-4 injection leads regularly to panic anxiety and rises in API and IDCL scores which has been described extensively in literature (Eser et al., 2007; Wiedemann et al., 2001). Because we were interested in very rapid effects concomitant with EMG alterations