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Electrophysiological Studies in Healthy Subjects Involving Caffeine

Mamede de Carvalho[a,b,*], Erica Marcelino[b] and Alexandre de Mendonça[b,c]
[a]*Department of Neurosciences, Hospital de Santa Maria, Lisbon, Portugal*
[b]*Instituto de Medicina Molecular, Lisbon, Portugal*
[c]*Laboratory of Neurosciences, Faculty of Medicine, Lisbon, Portugal*

Abstract. We review the electrophysiological studies concerning the effects of caffeine on muscle, lower and upper motor neuron excitability and cognition. Several different methods have been used, such as electromyography, recruitment analysis, H-reflex, transcranial magnetic stimulation (TMS), electroencephalography and event-related potentials. The positive effect of caffeine on vigilance, attention, speed of reaction, information processing and arousal is supported by a number of electrophysiological studies. The evidence in favor of an increased muscle fiber resistance is not definitive, but higher or lower motor neuron excitability can occur as a consequence of a greater excitation of the descending input from the brainstem and upper motor neurons. TMS can address the influence of caffeine on the upper motor neuron. Previous studies showed that cortico-motor threshold and intracortical excitatory and inhibitory pathways are not influenced by caffeine. Nonetheless, our results indicate that cortical silent period (CSP) is reduced in resting muscles after caffeine consumption, when stimulating the motor cortex with intensities slightly above threshold. We present new data demonstrating that this effect is also observed in fatigued muscle. We conclude that CSP can be considered a surrogate marker of the effect of caffeine in the brain, in particular of its central ergogenic effect.

Keywords: Caffeine, cortical silent period, lower motor neuron, muscle, transcranial magnetic stimulation

INTRODUCTION

Caffeine has effects on the motor system. These effects may be exerted at distinct levels of the nervous system, namely the muscle, the lower motor neuron, the upper motor neuron, or involve modulatory effects from other cortical areas.

MUSCLE

It has been described that caffeine has a positive ergogenic effect. This is supported by 3 different mechanisms. Firstly, alteration in fat metabolism, as caffeine promotes free fatty acid utilization through antagonism of adenosine receptors, sparing muscle fiber glycogen reserve [1]; secondly, a positive direct effect on calcium release from the sarcoplasmic reticulum by ryanodine receptors activation, a phenomenon readily observed in situ muscle preparation but with a non-physiological level of caffeine [2]; thirdly, increasing excitatory neurotransmitter activity as a consequence of adenosine receptor antagonism [3].

Caffeine has no positive effect on the power of the maximal muscle contraction, on the twitch contractile properties during exercise, or on the M-wave amplitude obtained by electrical stimulation of the peripheral nerve [4–8]. However, the positive effect of caffeine on endurance and fatigue is well demonstrated in a large number of studies [5,9–13]. It is not clear if this positive effect is related to a more effective muscular contraction. Detailed investigations in subjects asked to perform repeated submaximal contraction suggests

*Correspondence to: Professor M. de Carvalho, Department of Neurosciences, Santa Maria Hospital, Lisbon 1649-028, Portugal. Tel./Fax: +351 217805219; E-mail: mamedemg@mail.telepac.pt.

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that caffeine improves the sarcoplasmic calcium handling [5].

LOWER MOTOR NEURON

Caffeine antagonizes adenosine receptors at physiological doses, causing increased excitatory neurotransmitter release and lower neuronal activation threshold [3]. The augmented excitability of the serotoninergic neurons in the raphe nuclei can influence lower motor neurons (LMN) as they receive input from descending raphe fibers [14]. The monosynaptic Hoffman reflex (H reflex) is an indirect measure of the LMN excitability. Two studies found no change in the ratio H-reflex/M-wave amplitude in a healthy population after caffeine intake [9,15]. Moreover, the anticipated reduction of the H-reflex amplitude after exercise was not changed after caffeine ingestion [16]. However, another study detected significantly increased LMN excitability in 7 healthy controls after caffeine administration (6 mg/kg) by investigating the H-reflex stimulus-response curve [17]. Another approach is to explore F-wave amplitude and peripheral silent period to assess LMN excitability; however, in a healthy control population we did not find any change in those measurements after the ingestion of 200 mg of caffeine [18].

A different behavior of the LMN could change the recruitment pattern of the motor units during muscle contraction, especially in fatigue. There is no clear evidence that this occurs after caffeine consumption [5, 19]. Nonetheless, caffeine augments the incidence of self-sustained firing [20], which is a consequence of the increased LMN excitability and related to the presence of plateau potentials. Plateau potentials are facilitated by the tonic activity of descending serotoninergic and noradrenergic neurons [21]. This can be a protective mechanism in conditions causing muscle fatigue, as an increase in plateau potentials spares the necessary enlargement of the excitatory drive to LMN pools [20].

UPPER MOTOR NEURON

Transcranial magnetic stimulation (TMS) is the elective method to investigate non-invasively the cortical motor area function. The accumulated experience shows that caffeine does not change motor-evoked amplitude (MEP), central conduction time or corticomotor threshold [18,19,22].

A recent study in 11 healthy subjects disclosed no change of cortical excitability after caffeine (3 mg/kg), as evaluated by motor threshold (rest and active), short interval intra-cortical inhibition (SICI), intra-cortical facilitation (ICF), cortical silent period (CSP) (with intensities at 130, 150, 175% of active threshold) and size of the MEP (with intensities at 110%, 125% and 150% of rest motor threshold) [22]. The CSP refers to an interruption of voluntary muscle contraction by electrical or magnetic stimulation of the motor cortex. Although its first part, which is shorter, can depend on peripheral mechanisms related LMN inhibition, the larger last part depends on the inhibitory cortical interneurons. It is currently considered that the central component of the CSP depends on $GABA_B$ receptors, as concluded from TMS-pharmacological studies [23].

In a previous study, we tested 200 and 400 mg of caffeine in a group of healthy controls and confirmed that stimulus intensity 50% above cortical threshold did not modify CSP. However, applying an intensity of 10% above threshold, we observed a consistent and statistically significant decrease in the CSP (between 12–16%) in different upper limb muscles [18]. Although this phenomenon merits further research, we hypothesized that the inhibition of adenosine A_{2A} receptors could activate D2-dopamine striatal receptors, decreasing the activity of the GABAergic enkephalinergic striatopallidal neurons of the indirect pathway [18].

FATIGUE

The study of central fatigue with TMS is an exciting new area. The CSP lengthens and MEP increases during fatiguing contractions [24]. Immediately after exercise, MEP tested at rest shows increased amplitude as compared to the baseline response, a phenomenon termed post-contraction facilitation. After this period the MEP response is markedly depressed for as much as half an hour – long-lasting depression. This post-fatigue depression recovers rapidly during high-intensity muscle contraction. Some authors observed an increased post-activation potentiation after caffeine [19], but a similar fatigue-induced MEP depression. A more recent study shows that cortically-driven twitch can be increased by caffeine ingestion, although maximal voluntary activation is not altered during fatigue or recovery suggesting that voluntary activation is not limited by central excitability [25]. Keeping a mild contraction of the target muscle after exercise, the expected MEP depression is minimized after

caffeine, which represents caffeine-induced increased corticomotor excitability [25]. Further studies are recommended.

COGNITIVE FUNCTION

Caffeine increases serotonin concentration in supraspinal centers [14], noradrenergic neurons firing rates [26], and striatal dopamine release [27]. The striatum, the main input area of the basal ganglia, is rich in adenosine A_{2A} receptors, which are antagonized by caffeine. This interaction has large implications in the control of voluntary movement, as well as in motivational, emotional and cognitive aspects of motor behavior.

Low to moderate doses of caffeine have been reported to increase vigilance [28], attention and speed of reaction [29], information processing and sustained attention [30], sleep [31], and arousal [32].

Caffeine increases arousal as detected by arousal markers (increase in skin conductance and EEG alpha frequency, together with a global decrease in alpha power) [32]. However, the EEG changes are not striking and are possibly influenced by acute caffeine withdrawal or ingestion [33,34].

A number of studies using event-related brain potentials revealed that caffeine increases attention (N1, P2 and N2b responses), improves preparation (larger contingent negative variation), and increases arousal in fatiguing conditions (P3 responses) [30,35]. The action on attention and arousal can be modulated by dopaminergic pathways [30], but the arousal increase is also mediated by cholinergic neurons [36].

CORTICAL SILENT PERIOD IN FATIGUED MUSCLE: ORIGINAL DATA

AIM

Caffeine intake decreases CSP following TMS with intensity 10% above cortico-motor threshold [18]. Physiologically, CSP is significantly increased in the target muscle after fatiguing contraction [24,37]. Considering its ergogenic properties, we aimed to test if caffeine could reduce the CSP measured in a fatigued muscle. In addition, we intended to investigate the effect of caffeine on SICI and ICF in a rested target muscle, as described elsewhere [22], as well as on long-interval intra-cortical inhibition (LICI) in which caffeine influence was not tested so far.

METHODS

Population

Thirteen subjects (3 men, mean age 27.5 ± 3.3 years ranging from 20 to 31, mean weight 63.0 ± 7.9 from 53 to 75 kg) working at the Faculty of Medicine, University of Lisbon, were volunteers in this study. A structured questionnaire was applied to all subjects, in order to appraise socio-demographic characteristics and medical history, as well as caffeine exposure, alcohol consumption and smoking habits [18]. The inclusion criterion was age between 20 and 40 years, and no or moderate caffeine ingestion. The exclusion criteria were smokers, pregnant women, the presence of cardiac pacemakers, preceding neurosurgical intervention, history of epilepsy, and drug intake that could affect cortical excitability. All subjects gave informed consent and this protocol was approved by the local Ethics Committee.

Subjects were asked to abstain from caffeine-containing drinks and foods for at least 24 h before the study. At the same time of day (9 am), the participants underwent the experimental protocol. This study followed a cross-over design, in which the subjects received placebo (sucrose) or caffeine on the first day and the opposite option on the next session at least one week apart, as established by a previous randomization performed elsewhere. Participants and researchers directly involved in the experiment were blind to the nature of the substance. One hour after 200 mg of caffeine ingestion the electrophysiological tests were performed. The investigated caffeine dose (about 3.3 mg/kg for the mean weight of our population) has been used as the standard dose in other studies involving the central action of caffeine [18,22]. The capsule was prepared at the Hospital pharmacy and active and placebo capsules looked alike. At the end, subjects were asked to identify the experimental condition, caffeine or placebo.

Neurophysiology

A *Counterpoint* machine (Dantec, Skovlunde, Denmark) was used for electromyographic (EMG) and motor responses analysis. The temperature of the investigated limb was kept at or above 32 °C. Left ADM muscle was studied in each subject through surface electrodes (Ag-AgCl, recording area 7×4 mm) using a belly-tendon montage. Filtering was set 20 Hz–10 kHz for motor responses analysis and 5 Hz–1 kHz for surface EMG recording. Motor nerve conduction soft-

ware was used to investigate MEP amplitude, SICI, ICF, LICI and CSP. Proprietary software for spectral analysis of the EMG signal was used in this investigation. TMS was performed using a *MagPro* X-100 device (Medtronic, Skovlunde, Denmark); this device was equipped with a twin-mode facility to permit double-stimulation paradigms and a round stimulating coil with a monophasic pulse (coil winding diameter of 12 cm).

The center of the coil was positioned flat over the vertex, but moved as necessary to obtain a maximal response at the lowest stimulus intensity. We used clockwise electric flow in the coil for the right hemisphere stimulation. The subjects were asked to keep the hand relaxed during the investigation. Activation of motor units in the ADM was monitored through the surface electrodes by the audio system of the EMG device. We defined the resting MEP threshold in 5% increments of maximal stimulator output, from an initial stimulus intensity of 20%, as the minimum stimulus intensity that evoked at least 5 responses larger than 50 μV in 10 stimuli [18]. For recording the basal MEP amplitude the stimulus intensity was set at 2% above threshold. In each subject, 10 responses were obtained, with an inter-stimulus interval of at least 30 seconds, the mean value was considered as the basal response. To define SICI the conditioning stimulus intensity was set at 20% below threshold and the test stimulus at the threshold, with the inter-stimulus interval chosen at 4 ms. For ICF and LICI calculation both conditioning and test stimulus intensity were set at threshold, the inter-stimulus interval was established to 15 ms for ICF and 100 ms to LICI. With an interval of 5 seconds between trials 10 stimuli were applied for each condition and the mean amplitude calculated. For SICI, ICF and LICI the percentage of change was calculated (Table 1).

Following this first session in which the ADM muscle was relaxed, the subjects were asked to perform full maximal isometric contraction of the left ADM muscle for 20 seconds to record EMG signal, which was submitted online to power spectrum analysis to derive the median frequency of the signal. After 1 minute rest, the subjects were verbally stimulated to maintain a maximal isometric contraction of the left ADM muscle for 2 additional minutes. The maximal contraction was controlled by the interferential pattern on the screen of the EMG machine. At the end of this period and during contraction, a power spectrum analysis was again attained and the median frequency registered. In all subjects this protocol caused a marked feeling of fatigue of the contracting muscle. Power spectral analysis (median frequency) was performed to objectively test the efficacy of this protocol for muscle fatigue.

After a brief rest of 15 seconds following the 2 minutes period of maximal ADM contraction, the CSP was determined with TMS intensity 1.1 x threshold during ADM contraction. The duration of the CSP was defined as from the latency of the MEP response to the reappearing of the EMG activity (> 100 μV) in 10 superimposed non-rectified trials [18]. CSP duration as determined with this method was shown to be reliable and not dependent on the rater [18].

Analysis of the results was performed by one author of this paper (MdeC) blind to the nature of the capsule content.

Statistics

Statistical analyses were carried out with the Statistical Package for the Social Sciences (SPSS) software (version 16.0 for Windows). Values are given as mean $\pm$ standard deviation (SD). The two-tailed paired Student's t test was used to analyze the effects of caffeine on electrophysiological parameters in the motor cortex. Repeated measures ANOVA was used to study the effects of both fatigue and caffeine on the median frequency of the EMG electrical signal. The absence or presence of fatigue was considered in the within-subjects analysis, and the effect of caffeine in the between-subjects analysis. A possible interaction between fatigue and caffeine was also verified. Values of $p < 0.05$ were considered statistically significant.

RESULTS

The subjects were not able to differentiate caffeine *versus* placebo tablets ($p > 0.05$).

The contraction protocol applied in this study was effective in inducing fatigue as evaluated by subject symptoms and the change of the median frequency domain of the EMG electrical signal. Fatigue caused a decrease in the median frequency of the EMG interferential pattern from 85.6 $\pm$ 17.1 Hz to 60.0 $\pm$ 17.5 Hz before caffeine and a decrease from 78.9 $\pm$ 18.8 Hz to 58.9 $\pm$ 15.5 Hz after caffeine ($p = 0.001$); however, there was no effect of caffeine on the median frequency of the EMG electrical signal ($p = 0.13$) and no interaction between fatigue and caffeine ($p = 0.27$, repeated measures ANOVA, Table 1).

Remarkably, in spite of the persistence of the fatigue effect, caffeine could still decrease the CSP as

Table 1
Electrophysiological Results

	n	Before caffeine intake Mean ± SD (Min-Max)		One hour after caffeine intake Mean ± SD (Min-Max)		p value
		Rest	Fatigue	Rest	Fatigue	
Median Frequency (Hz)	13	85.6 ± 17.1 (66–122)	60.0 ± 17.5 (40–98)	78.8 ± 18.8 (62–135)	58.8 ± 15.5 (31–90)	0.13** **0.001***
CMT (%)	13	49.4 ± 5.5 (41–56)		48.6 ± 5.0 (41–59)		0.37*
MEP Amplitude (μV)	13	249.4 ± 183.0 (70–610)		304.5 ± 206.5 (82–646)		0.25*
SICI (4 ms) (%)	13	87.5 ± 16.3 (46.4–100)		83.8 ± 24.5 (21.8–100)		0.66*
ICF (15 ms) (%)	13	835.6 ± 1086.3 (−77.1–3542)		510.4 ± 421,5 (−82.5–1325,6)		0.33*
LICI (100 ms) (%)	13	77.7 ± 37.5 (−18.1–100)		48.99 ± 68.6 (−137.7–100)		0.20*
CSP (ms)	13		214.0 ± 43.7 (150–324)		177.5 ± 44.39 (110–251)	**0.004***

n – number of subjects; CMT – cortico-motor threshold; MEP – motor evoked potential; SICI – short inhibitory cortical interval (represents the % of the value of the baseline amplitude); ICF – intracortical facilitation (represents the % of the value of the baseline amplitude); LICI – long interval cortical inhibition (represents the % of the value of the baseline amplitude); CSP – cortical silent period (for definition see methods).
* Student's t test, comparison of the mean values before and after caffeine intake.
** Repeated Measurements ANOVA test, effect of caffeine (between-subjects analysis).
*** Repeated Measurements ANOVA test, effect of fatigue (within-subjects analysis).

previously shown under resting conditions (Table 1). Thus, when applying a stimulus intensity of 10% above threshold, the CSP obtained in fatigue conditions was decreased after caffeine intake (177.5 ± 44.4 ms), in comparison with the control value before caffeine (214.0 ± 43.7 ms, $p = 0.004$, Table 1). It should be added that, in experiments performed with the same subjects on separate days, the CSP obtained in fatigue conditions did not change significantly after placebo administration (196.6 ± 52.4 ms) in comparison with the value before placebo (203.3 ± 37.8 ms, $p > 0.05$).

Threshold, MEP amplitude, SICI, ICF and LICI were not significantly changed after caffeine intake (Table 1).

DISCUSSION

Threshold, MEP amplitude, SICI and ICF findings confirm previous results [18,22]. Threshold reflects the excitability of the motor neurons or of the associated interneurons; SICI and ICF results from the activity of the cortical interneurons as mediated by $GABA_A$ and glutamate receptors, respectively [38]. The effect of caffeine on LICI was addressed here for the first time; LICI represents cortical inhibition mediated probably by $GABA_B$ receptors [39]. Considering the common mediation by $GABA_B$ receptors of LICI and CSP, we might anticipate that both would show a parallel change following caffeine intake. However, opposite to CSP, the LICI did not change significantly after caffeine consumption. This can be explained by accepting that the modulation of both inhibitory phenomena is different [40], or hypothesizing that CSP is more sensitive than LICI to detect changes in $GABA_B$ receptor-mediated modulation. In this regard, it is interesting to consider again the effects of fatigue. Fatiguing contraction is known to reduce LICI whereas CSP increases in fatigued muscles [41]. This suggests that CSP duration and LICI may reflect processes occurring in different neuronal populations [41].

The fatigue protocol applied in this study was effective as confirmed by the significant decrease of the median frequency domain of the ADM electrical sign. This reflects the decrease in the muscle fiber conduction velocity [42], and the synchronization of the motor units [43]. However, no positive effect of caffeine could be observed on the electrical activity of the muscle using this simple protocol [4].

Investigating a fatigued muscle, we confirmed that CSP is significantly shorter after caffeine consumption; the magnitude of CSP reduction (17%) was similar to the one observed before in a rested muscle [18]. Experimental manipulation of $GABA_B$ receptors, which are inhibitory autoreceptors, is known to modulate the CSP [23,44]. In this context, caffeine might interfere with GABAergic neurotransmission in different

ways [18]. It was proposed that caffeine, by antagonizing adenosine A_{2A} receptors, could inhibit the release of GABA, thus reducing GABAergic inhibitory transmission in the motor cortex, and hence decreasing the CSP [18] Experimental support for this possibility comes from the excitatory effect for adenosine A_{2A} receptors described on GABA release in the hippocampus [45]. However, the alternative possibility that caffeine could decrease the CSP by modifying the properties of extrinsic cortico-basal-thalamo-cortical pathways that control motor cortex activity could not be ruled out. Adenosine A_{2A} receptors are found in brain areas rich in dopamine such as the basal ganglia, where they are associated with D_2 dopamine receptors [46, 47], and any significant changes induced by caffeine within the basal ganglia would very likely exert a downstream effect on the motor cortex.

CONCLUSIONS

Caffeine has been extensively studied for its positive effects on fatigue. It is possible that a direct effect on muscle fiber or lower motor neuron is relevant [5,9]. On the other hand, the central effect is large and well documented [19,27–34]. CSP as estimated using low intensities is a sensitive method to detect the influence of caffeine on the central nervous system [18]. Our present results confirm that CSP is a potential surrogate marker of this influence. The fact that this change is also observed in fatigued muscles suggests that CSP can measure the central ergogenic action of caffeine.

DISCLOSURE STATEMENT

Authors' disclosures available online (http://www.j-alz.com/disclosures/view.php?id=273).

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