Power spectral analysis of electromyographic and systemic arterial pressure signals during fentanyl-induced muscular rigidity in the rat


SUMMARY

We have measured electromyographic (EMG) and systemic arterial pressure (SAP) signals during fentanyl-induced muscular rigidity in adult male Sprague-Dawley rats anesthetized initially with ketamine 120 mg kg$^{-1}$ i.p. during controlled ventilation. Fentanyl 100 µg kg$^{-1}$ i.v. induced significant increase in EMG activity, recorded from the sacrococcygeus dorsi lateralis muscle. Power spectral analysis revealed that this was produced by an increase in the root mean square and a decrease in the mean power frequency values of the signals, signifying recruitment and synchronous activation of motor units. Together with transient hypotension and bradycardia, power spectral analysis of the SAP signals demonstrated a reduced but maintained power density of the frequency components that represent respiratory, baroreceptor and vasomotor activities. All these effects were only demonstrated unequivocally in rats maintained by i.v. infusion of ketamine until 10 min before the administration of fentanyl. We conclude that analysis of the temporal alterations in the spectral components of the EMG and SAP signals in rats during mechanical ventilation provides a sensitive method of measuring fentanyl-induced muscular rigidity and the accompanying alterations in haemodynamic variables. (Br. J. Anaesth. 1994; 72: 328-334)

KEY WORDS


Administration of large doses of fentanyl [1] in cardiac anaesthesia is now common clinical practice [2-4]. Unfortunately, it is accompanied commonly by muscular rigidity, especially during induction of anaesthesia [3, 5, 6].

In the search for the precise mechanism(s) that underlies opioid-induced muscular rigidity, an approach used frequently [7-10] as the experimental model, first reported by Wand, Kuschinsky and Sontag [7], is electromyographic (EMG) activity in unanaesthetized, spontaneously ventilating animals. However, this model contains confounding factors including hypoventilation, hypoxaemia, hypercapnia and bradycardia, all of which are associated commonly with large doses of opioids [2, 5, 6]. In 1989 [11], we described a modified approach in the evaluation of fentanyl-induced muscular rigidity. We used integrated EMG activity in gastrocnemius and rectus abdominis muscles in rats anaesthetized with ketamine during mechanical ventilation and we identified the active participation of coeruleospinal noradrenergic pathways in the muscular rigidity produced by fentanyl [12, 13].

Three recent developments in our laboratory formed the immediate background for the present study. First, we found that the EMG signals from the sacrococcygeus dorsi lateralis (SCDL) muscle, in comparison with those of gastrocnemius and rectus abdominis muscles [13], are an even more sensitive quantitative index for muscular rigidity. Second, we obtained the technology [14, 15] to carry out simultaneous, continuous, online and real-time power spectral analysis of EMG and systemic arterial pressure (SAP) signals. Third, we observed recently that the method of delivering the maintenance dose of ketamine may affect the outcome of fentanyl-induced muscular rigidity in our animal model. We report here that analysis of the temporal alterations in the spectral components of the EMG (e.g. from the SCDL muscle) and SAP signals in rats undergoing mechanical ventilation and maintained by i.v. infusion of ketamine until 10 min before administration of fentanyl provides a sensitive method to quantify fentanyl-induced muscular rigidity.

METHODS

General preparations

The procedures used in this study have been approved by the Experimental Animal Committee of National Yang-Ming Medical College. We used adult male Sprague-Dawley rats (230-300 g), anaesthetized initially with ketamine 120 mg kg$^{-1}$ i.p. The animals underwent mechanical ventilation to maintain the end-tidal carbon dioxide concentration within 4-5%. The right femoral artery and vein were cannulated for measurement of SAP and
administration of drugs, respectively. Rectal temperature of animals was maintained at $37 \pm 0.5^\circ C$ throughout the experiment with a heating pad.

EMG signals were recorded differentially using a pair of platinum needle electrodes (Grass Type E2) inserted into the left SCML muscle. The bioelectric signals were amplified and filtered (frequency range 3–1000 Hz) by an universal amplifier (Gould G-20-4615-58). This response range covered the range of EMG frequencies (3–500 Hz) in which we were interested.

The arterial catheter (Clay Adams PE-50) was connected to a pressure transducer (Statham P23ID, frequency range DC–200 Hz) and thence to a pressure processor amplifier (Gould G-20-4615-52) from which the SAP signals were amplified and filtered (frequency range DC–100 Hz). The response of our transducer–preamplifier was within the frequency range (0–3 Hz) of interest in our spectral analysis of the SAP signals.

Simultaneous, continuous, online and real-time power spectral analysis of EMG and SAP signals

EMG and SAP signals were displayed throughout the experiment on a polygraph (Gould RS 3400). The same signals were also digitized (Neurocorder DR–484) and stored on videotape. In addition, they were subjected to simultaneous, continuous, online and real-time power spectral analysis, using a computer algorithm developed by our laboratory [14, 15].

We used a general purpose IBM-PC compatible personal computer equipped with an Intel 80486 DX microprocessor. For online spectral analysis, both
EMG and SAP signals were relayed simultaneously to an analogue-to-digital converter (Advantech PCL-818) connected to the computer, and were digitized at a rate of 2048 Hz.

The digitized signals to be analysed were truncated into small time segments (windows). For each time segment, our algorithm first estimated the power density of the spectral components based on fast Fourier transform [16]. It subsequently quantified the magnitude of EMG activity by calculating the root mean square (RMS) value, and evaluated the frequency domain of the EMG signals by calculating the mean power frequency (MPF) of each spectrum. These two values represent, respectively, the number of active motor units and the degree of their synchronization during muscle contraction [17, 18].

Our algorithm also analysed the spectral components of the SAP signals. In this regard, we were particularly interested in the low frequency components of the SAP spectrum. They included the very low frequency (VLF 0.00-0.25 Hz), low frequency (LF 0.25-0.90 Hz) and high frequency (HF 0.90-1.70 Hz) components. These three spectral components purportedly reflect the influence of vasomotor tone, baroreceptor activity and respiration [19,20] on SAP. Each component was quantified by the method of integration, that is calculation of the area of power spectral density between two specified frequencies.

Raw EMG and SAP signals, their respective two-dimensional spectrogram, together with heart rate and values of RMS, MPF and every SAP spectral component calculated for each time segment were displayed either graphically or numerically on a monitor, printer, or both. By repeating these procedures continuously, we were able to examine the simultaneous spectral changes of EMG and SAP signals over time in a real-time and online manner.

Experimental protocols

All recording sessions took place in a quiet room with minimal physical disturbance. In addition to studying the effects of fentanyl on the EMG and SAP signals, we also evaluated if the outcome of these effects was affected by the method of delivering the maintenance dose of ketamine. Thus one group of animals was given hourly bolus supplements of ketamine 60 mg kg\(^{-1}\) i.p.; the second group was maintained with an i.v. infusion of ketamine 30 mg kg\(^{-1}\) h\(^{-1}\) until 10 min before administration of fentanyl. In all experiments, continuous, online and real-time power spectral analysis of EMG and SAP signals were carried out before and at least 20 min after i.v. administration of fentanyl 100 \(\mu\)g kg\(^{-1}\).

Statistical analysis

The digital output of the results from computer analysis of EMG and SAP signals for each time segment (64 s for EMG and 32 s for SAP) was collected for data analysis. The averaged value for each 5-min interval was used for statistical evaluations. We used two-way analysis of variance (ANOVA) with repeated measures to assess the group difference between the two methods of delivering ketamine supplements. This was followed by the Student–Newman–Keuls multiple range test for a posteriori analysis of individual means at various time points. We also used the Dunnett test to analyse the effect of fentanyl vs preinjection control. \(P < 0.05\) was taken to indicate statistical significance.

RESULTS

Effects of fentanyl on the spectral components of EMG and SAP signals

Figure 1 illustrates an example of simultaneous, continuous, online and real-time spectral analysis of EMG and SAP signals after administration of fentanyl to an animal anaesthesized with an i.v. infusion of ketamine. We observed that together with the greatly increased raw EMG activity, fentanyl 100 \(\mu\)g kg\(^{-1}\) i.v. induced an increase in the RMS and a decrease in MPF values. More interestingly, whereas grossly observed SAP or heart rate signals showed only minor and transient hypotension...
Fentanyl and EMG-SAP power spectrum

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Fig. 3. Effects of fentanyl 100 μg kg⁻¹ i.v., given at time 0, on mean systemic arterial pressure (MAP) and heart rate (HR) in animals maintained with an i.v. infusion (O) of ketamine until 10 min before administration of fentanyl or by i.p. hourly bolus supplement (#). Values at each time point are mean and SEM of data recorded over 5 min (n = 6 animals per group). Significant differences (P < 0.05) between the two groups (ANOVA): *P < 0.05 compared with before administration of fentanyl (Dunnett test).

Influence of the method of delivering ketamine supplements

We found also that the outcome of the above-mentioned effects of fentanyl was affected partially by the method of delivering the dose of ketamine. Whereas a significant increase in EMG RMS was observed in both groups (fig. 2), appreciable reduction in EMG MPF was manifested only in animals given an i.v. infusion of ketamine until 10 min before administration of fentanyl. Furthermore, the former group exhibited greatly increased basal MPF values (fig. 2).

Fentanyl 100 μg kg⁻¹ i.v. induced significant transient hypotension and bradycardia (fig. 3), and an appreciable decrease in the HF component (fig. 4) in animals maintained with an i.v. infusion of ketamine. The basal value of the HF component of SAP signals and bradycardia, online spectral analysis revealed an overall reduction in VLF, LF and HF components of the SAP signals.

Fig. 4. Effects of fentanyl 100 μg kg⁻¹ i.v., given at time 0, on the high (HF), low (LF) and very low (VLF) frequency components of systemic arterial pressure (SAP) signals in animals maintained with an i.v. infusion (O) of ketamine until 10 min before administration of fentanyl or by i.p. hourly bolus supplement (#). Values at each time point are mean and SEM of data recorded over 5 min (n = 6 animals per group). Significant differences (P < 0.05) between the two groups (ANOVA): *P < 0.05 compared with before administration of fentanyl (Dunnett test); †P < 0.05 compared with i.p. bolus group (Student-Newman-Keuls test).

was enhanced particularly in this group (fig. 4), resulting in a reduction after i.v. fentanyl. The VLF component, however, was diminished in animals maintained by both methods of delivering ketamine (fig. 4). Of note, despite the overall reduction, was that these frequency components of SAP signals still maintained sufficient power (VLF 43.9%, LF 16.1%, HF 31.2%).
FIG. 5. Illustrative example of simultaneous, continuous, online and real-time analysis of cardiovascular and electromyographic (EMG) activities after i.v. administration of saline (arrow) to a rat maintained with an i.v. infusion of ketamine. Systemic arterial pressure (SAP), heart rate (HR) and online three-dimensional power spectrogram showing successive power spectral density (PSD) of SAP signals over 25 min are displayed. Also shown are temporal alterations of the integrated values for the high frequency (HF), low frequency (LF) and very low frequency (VLF) components of the spectra. Changes in EMG signals, the corresponding three-dimensional spectrogram showing the successive PSD of these signals, root mean square (RMS) and mean power frequency (MPF) were also monitored continuously for 25 min. Window length: 32 s with no overlapping for SAP and 64 s with 50% overlapping for EMG signals.

The time interval between cessation of i.v. infusion of ketamine and administration of fentanyl was found to be crucially important. An interval shorter than 10 min produced equivocal results. In contrast, animals began to recover from ketamine anaesthesia (movement of the head, extremity or tail) [11] when the time interval was more than 10 min. One question that arises is whether or not the increase in EMG activity 10 min after cessation of ketamine may simply reflect recovery from anaesthesia and therefore has no relationship to fentanyl. That this may not be the case is illustrated in figure 5. As shown, administration of saline 10 min after cessation of ketamine resulted in only sporadic EMG activation, simultaneous with an intermittent increase in RMS and, interestingly, MPF values. Online spectral analysis also revealed a slow increase in the HF, LF and VLF components of the SAP signals, signifying gradual emergence from anaesthesia.

DISCUSSION
We have found that i.v. administration of fentanyl induced a significant increase in the EMG activity of the SCGL muscles. Further computer analysis revealed that this was accomplished by an increase in the RMS and a decrease in the MPF values of the signals. In addition to transient hypotension and bradycardia, power spectral analysis of the SAP signals demonstrated a reduction in the HF, LF and VLF components. We found also that these effects
FENTANYL AND EMG–SAP POWER SPECTRUM

were unequivocally and significantly demonstrated only in rats maintained by i.v. infusion of ketamine until 10 min before administration of fentanyl.

A traditional way of analysing physiological signals is to assess the amplitude of these signals, often alterations of magnitude in a time domain. In contrast, analysis of the frequency domain of physiological signals has not received attention until recently. During the past few years, quantification of EMG signals by RMS and MPF values has gained acceptance as a sensitive method that offers valuable information on the physiological and pathological characteristics of skeletal muscle contraction [17, 18, 21, 22]. Similarly, power spectral analysis of SAP signals is considered a highly efficient procedure for evaluation of cardiovascular function [19, 20]. Indeed, with the newly developed capability for simultaneous analysis of the power spectrum of EMG and SAP in a continuous, online and real-time basis [14, 15], the present study revealed several hitherto unidentified features that were associated with administration of fentanyl.

Several authors have reported that muscular rigidity induced by opioids is associated with an increase in the RMS values of EMG activity [9, 10], which signifies recruitment of active motor units [17, 18]. However, to increase muscle power effectively, this process must be accompanied by synchronous activation of these motor units. Thus it is intriguing to note that the increase in the RMS values of the SCDL muscles was associated with a decrease in MPF values, which denotes a concerted action of the individual motor units [17, 18]. That the decrease in MPF values was particularly conspicuous in animals that received an i.v. infusion of ketamine lends further support for the latter concept. The MPF value before fentanyl administration in this group was approximately 40% greater than that in animals who received hourly bolus supplements of ketamine. This suggests that the motor units in the former group were activated from a much more asynchronous state after administration of fentanyl.

Our power spectral analysis of the SAP signals also revealed interesting haemodynamic effects of fentanyl. The temporal alteration of the HF component, which represents respiratory efforts [19, 20], reflects the interplay between mechanical ventilation, emergence from ketamine anaesthesia and fentanyl. An increase in resistance to mechanical ventilation as the animal emerged from ketamine anaesthesia was manifest by the heightened HF component. This was reduced substantially after administration of fentanyl, which is known to cause respiratory depression [5, 6]. The greatly diminished LF component suggests that fentanyl suppressed the influence of baroreceptor afferents on cardiovascular function [19]. The preservation of sufficient power of the VLF component, which suggests a relatively maintained vasomotor tone [19], provides an explanation for the well-documented cardiovascular stability of fentanyl [2, 4]. That both LF and HF components were not suppressed completely further suggests that animals influenced by fentanyl still maintain a certain degree of cardiovascular and respiratory regulatory capabilities.

Ketamine is unique in producing an unusual trance-like state known as dissociative anaesthesia [23, 24]. We have observed previously that animals anaesthetized with this agent respond to fentanyl with an increase in EMG activity [11] but, presumably because ventilation is controlled, fail to exhibit hypercapnia and acidosis. We also found in the present study that the effect of fentanyl was influenced also by the methods of delivering the maintenance dose of ketamine. Animals given an hourly bolus injection of ketamine may be at varying levels of anaesthesia before they received fentanyl. On the other hand, animals that were allowed to emerge from ketamine anaesthesia 10 min before administration of fentanyl appeared to be in a more uniform, but light state of anaesthesia. The latter state was reflected by a heightened MPF value of the EMG, and HF component of the SAP, signals.

It is possible that the increase in EMG activity in response to fentanyl, given 10 min after cessation of i.v. infusion of ketamine, may be caused either by the opioid or by recovery from anaesthesia. Nonetheless, these two forms of EMG excitation are physiologically different. As was demonstrated clearly by the simultaneous increase in MPF values, EMG activation during emergence from ketamine anaesthesia (fig. 5) was intermittent, signifying asynchronous discharge of motor units. In contrast, administration of fentanyl (fig. 1) produced intensified EMG RMS activity accompanied by a decrease in MPF values, indicating synchronous discharge of motor units. Furthermore, the alteration in the spectral components of SAP signals with fentanyl was substantially different from that during emergence from ketamine anaesthesia.

In summary, we have found that subtle changes that contain important physiological information in EMG and SAP signals induced by fentanyl may be detected by subjecting these signals to power spectral analysis. Thus our study may provide a sensitive method of quantifying fentanyl-induced muscular rigidity and the accompanying alterations in haemodynamic variables. Based on this model, we found recently [25] that a pertussis toxin-sensitive guanine nucleotide–binding regulatory protein(s), which is not likely to be Gs, possibly Gi, Go or Gp, may be involved in the signal transduction process that underlies the muscular rigidity induced by fentanyl via an action on the locus coeruleus.

ACKNOWLEDGEMENTS

Supported in part by research grants NSC81-0412-B075-59 (TYL) and NSC82-0412-B010-010 (SHHC) from the National Science Council, Taiwan, Republic of China.

REFERENCES


