Fentanyl increases catecholamine oxidation current measured by *in vivo* voltammetry in the rat striatum

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A proposed mechanism for fentanyl-induced muscular rigidity is the effect of opioids on dopaminergic transmission in the striatum. The objective of this study was to observe the effect of fentanyl on the rat striatal catechol oxidation current (CA.OC) which reflects extracellular DOPAC (3,4-dihydroxyphenyl-acetic acid) concentration (a major metabolite of dopamine), as measured by in vivo voltammetry. Male Sprague-Dawley rats, anaesthetized with halothane, were stereotactically implanted with carbon fibre electrodes in the striatum and after an initial stabilization period of an hour were given a control saline IV injection followed 30 min later by fentanyl $10 \mu\text{g} \cdot \text{kg}^{-1}$ IV over 10 min and at 70 min by the monoamine oxidase inhibitor pargyline $70 \text{ mg} \cdot \text{kg}^{-1}$ IP. Fentanyl produced a significant ($P < 0.05$ Anova) increase in CA.OC in all animals. This reached a plateau 15 min following the administration of fentanyl and was at a maximum of 148 ± 10.2 per cent of control 35 min after the administration of fentanyl. Pargyline produced a rapid decline in CA.OC peak height which went from 143 ± 11.6 to 39 ± 6.8 per cent of control over 30 min. There were no significant differences between the pH, PaO_2 and PaCO_2 during the saline and fentanyl injection periods and there was no significant variation of blood pressure throughout the experiment. This study shows that under stable physiological conditions, fentanyl produces a significant increase in CA.OC in the rat striatum.

Key words

ANAESTHETICS, INTRAVENOUS, fentanyl; MEASUREMENT, TECHNIQUES: voltammetry; NEUROPHARMACOLOGY: striatum; SYMPATHETIC NERVOUS SYSTEM: catecholamine, dopamine.

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Supported by the MRC of Canada and Centre National de la Recherche Scientifique, France (CNRS UMR12).

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Chest wall rigidity following administration of opiates such as fentanyl is a problem which may lead to ventilatory difficulty.¹⁻³ The cause of this opiate-induced muscle rigidity is not understood and includes hypotheses of spinal and supraspinal mechanisms. One proposed mechanism of fentanyl-produced muscular rigidity is the effect of opioids on dopaminergic transmission in the striatum.⁴ This region contains the highest density of dopamine-containing neuronal terminals in the brain and is also the largest catecholamine-containing area in the brain with an almost exclusive dopaminergic innervation.⁵ There have been discrepant reports showing that striatal dopamine levels increase, decrease or remain constant following opiate administration.⁶⁻¹¹ This discrepancy may be due to differences between *in vitro* and *in vivo* experiments, the means of detecting and stimulating dopamine release, or species. Also, lack of physiological control in the *in vivo* experiments may cause hypo/hypertension, hypo/hypercapnia and hypoxia which may effect brain catecholamine metabolism.¹²⁻¹³ The objective of this study was to observe the effect of fentanyl on the rat striatal catechol oxidation current, which reflects dopamine metabolism,¹⁴⁻¹⁹ as measured by *in vivo* voltammetry during normotension, normocapnia and with a $\text{PaO}_2 > 100 \text{ mmHg}$.

Methods

Male rats (Sprague-Dawley OFA strain, 500–600 g) were housed two per cage in the laboratory (light 7 AM–7 PM, temperature $22^\circ \pm 1^\circ \text{C}$) for at least three days before the experiment. Rat chow and tap water were given *ad libitum*.

Anaesthesia was induced and maintained with halothane and air/oxygen. A tracheostomy was done and the animals mechanically ventilated (Harvard 50-1718, rate = $48 \cdot \text{min}^{-1}$, oxygen concentration 40–50 per cent, inspired halothane 0.75–1.5 per cent) to maintain an end-tidal PCO_2 (Engstrom Eliza Duo modified with a neonatology kit) at $40 \pm 3 \text{ mmHg}$. Rectal temperature was maintained at $38 \pm 0.5^\circ \text{C}$ with a Harvard warming blanket. As soon as possible after induction of anaesthe-

sia, a colloid (dextran 40,000 MW, in saline, Rheomacrodex) Na bicarbonate (1 M, 1.5 ml per 20 ml of dextran preparation) and glucose (30 per cent with 1.0 ml per 20 ml of dextran preparation) solution was continuously (Harvard Electronic syringe 568 816) infused ($7 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$) by a femoral venous catheter. Arterial pressure was continuously measured through a femoral arterial catheter with a Gould transducer and a Gould transducer amplifier 8800.06 recorder. Skeletal muscle relaxation was produced with metocurine $200 \mu\text{g} \cdot \text{kg}^{-1}$, vecuronium $50 \mu\text{g} \cdot \text{kg}^{-1}$, after ensuring an adequate level of anaesthesia upon nociceptive stimulation. Only animals which had minimal blood loss $< 1\text{--}2 \text{ ml}$ during vascular catheterization and surgery were included in these experiments. Arterial blood samples ($85 \mu\text{l}$) were analyzed for pH and blood gas estimation within 2 min on an ABL 300, acid-base laboratory, Radiometer, Copenhagen.

Rats were placed prone in a stereotaxic frame with the incisor bar 10 mm below the interaural line. The skull and dura were opened. The cortical surface was carefully cleaned with physiological saline. The pia was punctured by means of a used carbon fibre electrode. The treated carbon fibre electrode¹⁴ was implanted through this hole into the striatum at the following coordinates (A - 1 mm, L - 2.3 mm and depth from the brain surface 4.3 mm). The electrode was composed of carbon fibre insulated by a glass micropipette with the active surface being an exposed pyrolytic carbon fibre, $8\text{--}12 \mu\text{m}$ in diameter and $500 \mu\text{m}$ in length. Auxilliary and reference electrodes were in contact with the skull by means of a semi-liquid junction.

Differential normal pulse voltammetry was performed (Biopulse, Tacussel, Villeurbanne) as described¹⁵ every minute *in vitro* and *in vivo*. Carbon fibre electrodes MFC1 (Tacussel), were treated *in vitro* and tested in a test solution, PBS, (pH 7.4) ascorbic acid (AA, $200 \times 10^{-6} \text{ M}$, Sigma), dihydroxyphenylacetic acid (DOPAC $20 \times 10^{-6} \text{ M}$, Sigma). The catechol oxidation current (CA.OC) was identified as a peak appearing at $+55 \text{ mV}$ *in vivo* and *in vitro*. This technique allowed detection of DOPAC within the range of $0\text{--}60 \mu\text{M}$ (physiologic levels $22 \pm 4 \mu\text{M}$, mean $\pm$ SD).¹⁷ Electrodes in these experiments allowed stable recording of the CA.OC for periods in excess of the duration of the study.

In preliminary experiments the effect of fentanyl ($5\text{--}100 \mu\text{g} \cdot \text{kg}^{-1}$ IV) was studied. Doses $> 10 \mu\text{g} \cdot \text{kg}^{-1}$ produced significant hypotension and bradycardia and thus it was decided to use a dose of $10 \mu\text{g} \cdot \text{kg}^{-1}$ in the definitive experiment, to maintain cardiovascular stability. In addition, continuous administration of halothane proved superior as an anaesthetic technique to intermittent IV boluses of chloralose following induction with chloral hydrate regarding maintenance of cardiovascular stabil-

FIGURE 1 Effect of fentanyl and pargyline on the striatal catecholamine oxidation current. Results are expressed as percentage change from baseline recordings of peak height. Time is expressed in min following saline, fentanyl and pargyline injection respectively. Asterisks represent significant change ($P < 0.05$).

ity. Atropine $0.01 \text{ mg} \cdot \text{kg}^{-1}$ was administered IV prior to fentanyl to decrease the vagal effect of this drug. This dose was found to have no effect on the CA.OC in preliminary experiments. In seven rats, following initial instrumentation and implantation of the electrode, an initial stabilization period was allowed of one hour, until the peak CA.OC height varied less than ten per cent. Following this the control IV saline injection was administered and the CA.OC recorded for 30 min. After this period fentanyl $10 \mu\text{g} \cdot \text{kg}^{-1}$ was administered over 10 min and the CA.OC observed over 30 min. Subsequently the monoamine oxidase inhibitor pargyline, $70 \text{ mg} \cdot \text{kg}^{-1}$, was injected IP to observe the decay of the CA.OC. Following the experiment an electrolytic lesion (direct current $+5$ volts for 5 sec) was made using the working electrode, then the brain was rapidly removed for histological processing with cresyl violet staining.

Results of the CA.OC were expressed as a percentage of the mean control value calculated by averaging the five absolute values of CA.OC peak height obtained during the last 10 min prior to injection. Results were expressed as mean $\pm$ standard error. Statistical analysis of the CA.OC and blood pressure data was performed using repeated measures analysis of variance followed by the Newman-Keuls test for significant F-ratio. Arterial blood gas data were analyzed using the paired Student's *t* test. Significance was assessed at the $P < 0.05$ level.

Results

Fentanyl produced a significant increase in the CA.OC in all animals (Figure 1). This reached a plateau 15 min after the administration of fentanyl and was at a maximum of 148 ± 10.2 per cent of control, 35 min after injection.

FIGURE 2 A photograph of the right cerebral hemisphere obtained from a histological section. The carbon fibre was destroyed *in situ* by application of DC/+5V potential and demonstrates a lesion in the striatum around the tip of the electrode as well as the track of the fibre through the brain.

Administration of pargyline produced a rapid decline in CA.OC peak height which decreased from 143 ± 11.6 per cent to 39 ± 6.8 per cent of baseline over 30 min with a half life of 12.6 min. There was no significant change in systolic blood pressure throughout the experiment although blood pressure was decreased after pargyline. In addition there were no significant differences in pH and blood gases during the saline and fentanyl study periods: pH - 7.36 ± 0.01 , PO_2 - 254 ± 16.0 , PCO_2 - 37.7 ± 0.7 mmHg (saline), pH - 7.36 ± 0.01 , PO_2 - 258 ± 13.0 , PCO_2 - 37.1 ± 0.6 mmHg (fentanyl). Histology (Figure 2) confirmed that the site of the electrode tip was within the striatum.

Discussion

The development of *in vivo* electrochemistry in the study of brain neurochemistry has allowed the investigation of the nigrostriatal dopamine system.¹⁶⁻¹⁸ In *in vivo* voltammetry, an applied potential is used to cause oxidation of chemical species. The resulting current is then measured and related to the molecular species of interest in the extracellular fluid of the brain. Using a microelectrode implanted in the rat brain, this technique measures continuously the current generated by the loss of electrons of various biological molecules such as the catechola-

mines or their metabolites. As catecholamines are easily oxidizable, they have been extensively studied with the use of treated carbon fibre electrodes and differential normal pulse voltammetry. Following release, dopamine is metabolized to DOPAC by the enzyme monoamine oxidase. The main catechol present in the extracellular space in the vicinity of the dopaminergic terminals is DOPAC. In fact, DOPAC concentrations there are 200-1000 times higher than that of dopamine. Unfortunately, treating the carbon fibre electrode does not distinguish between DOPAC and dopamine since both catecholamines oxidize at almost the same potential. Electrical stimulation of dopaminergic pathways induces an increase in dopamine release followed by an increase in extracellular DOPAC levels.¹⁹ It is accepted that it is not dopamine, the neurotransmitter released from the nerve terminals, but its major breakdown product DOPAC, which contributes to the CA.OC peak.¹⁷ Thus voltammetry is a technique which has an advantage compared with classical biochemical methods of enabling a continuous analysis of DOPAC, the main dopamine metabolite *in vivo*.⁵ This study demonstrates that fentanyl causes a significant increase in the CA.OC which reflects an increase in the extracellular concentration of DOPAC resulting from an increased dopamine turnover in the striatum.

There is controversy concerning the effects of opiates on striatal dopamine release. Various reports that striatal dopamine levels increase, decrease, remain constant or have a biphasic response following opiate administration are available.⁶⁻¹¹ Concerning *in vivo* studies, the effect of opiates on arterial blood pressure, oxygenation, and carbon dioxide retention may possibly affect catecholamine metabolism. For example, recording of catechol oxidation current in the substantia nigra, a dopaminergic group which is not involved in cardiovascular regulation, during hypotension produced a delayed increase in the CA.OC to 125 ± 5 per cent of baseline.¹³ With the ultimate physiological derangement, lack of cerebral perfusion, immediately following overdose, there is a massive increase in CA.OC measured in the rat striatum. Thus, because opiates were reported to both increase and decrease dopamine release from the rat striatum, these experiments were done to detect *in vivo*, on line CA.OC, in the rat striatum under stable physiological conditions following fentanyl administration.

These results show that fentanyl causes an increased CA.OC in the rat striatum. This is caused by an increase in extra-cellular DOPAC, the main dopamine metabolite, which reflects increased dopamine turnover. Subsequent administration of the monoamine oxidase inhibitor pargyline, which inhibits DOPAC synthesis induced a rapid decline in the CA.OC identifying its catechol nature with a similar DOPAC half life to that previously reported.¹⁷

Previous studies have found varying effects of fentanyl on striatal dopamine. In halothane anaesthetized cats with cannulae in the caudate nuclei and substantia nigrae, fentanyl produced a biphasic increase followed by a delayed inhibition of tritiated dopamine release²⁰ although there was no recording of arterial pressures during these experiments. Our study may have missed a biphasic response as experimental time was minimized in order to decrease the effect of time on electrode deterioration. *In vitro* studies of pooled rat striata showed that fentanyl in low doses ($0.02\text{--}0.05\text{ mg}\cdot\text{kg}^{-1}$) had no significant effect on dopamine turnover 20 min after IP injection and raised the homovanillic acid (an o-methylated metabolite of dopamine) concentration in the rat striatum with a maximum at 60 min after IP injection. Higher doses ($0.1\text{--}0.2\text{ mg}\cdot\text{kg}^{-1}$) reduced the homovanillic acid content after 20 min and raised it after 60 min.²¹ Fentanyl was, in addition, found to be without effect on spontaneous release of H3 dopamine continuously formed from H3 tyrosine studied in rat striatal slices.²²

Dopamine is an important neurotransmitter involved in the control of movement. Patients with Parkinson's disease have a dopamine deficiency in the caudate and putamen of the striatum and have characteristic muscular rigidity.²³ Similarly, it has been hypothesized that fentanyl-induced rigidity may be the result of its effect on striatal dopaminergic transmission.⁴ The effect of fentanyl on increasing the CA.OC, which reflects dopamine release in the rat striatum demonstrated in this study may be the result of a variety of potential manipulations of the dopaminergic striatal circuitry. Opiates may act at presynaptic opiate receptors in the striatum which contain dopamine autoreceptors that prevent dopamine release when activated, or may produce different indirect effects at dopamine receptors depending on opiate ligand types. The μ receptor agonist opiates may act at presynaptic sites on nigrostriatal dopamine neurons activating dopamine autoreceptors to prevent dopamine release whereas delta ligands may act on dopamine nerve terminals in the striatum to stimulate dopamine release.¹¹ If the rigidity caused by fentanyl is proposed to be due to a decreased release of striatal dopamine as in a Parkinson-like syndrome, then one could expect a decrease in CA.OC in the striatum. The results of this study are at variance with this and in fact show an increase in CA.OC following fentanyl. On the other hand, *in vivo*, impaired dopaminergic transmission is accompanied by an increased synthesis and turnover of dopamine such as is postulated to occur after blockade of dopamine receptors by neuroleptics of the phenothiazine or butyrophenone type. That opiates such as fentanyl cause a blockade of dopamine receptors similar to the phenothiazines is unlikely¹⁰ and perhaps the opiate-induced rigidity caused by fentanyl may be due to

actions at other brain structures such as those surrounding the nucleus raphe pontis.²⁴

In summary, since fentanyl-induced muscular rigidity has been postulated to result from changes in striatal dopaminergic transmission and there are discrepant observations on the effects of opiates on the rat striatum, we studied by *in vivo* voltammetry the effects of fentanyl on the CA.OC in the rat striatum under optimized physiological conditions. Fentanyl produced a significant increase in the CA.OC which measures extracellular DOPAC, a metabolite of dopamine, which was abolished by the MAO inhibitor pargyline. Experiments were performed while maintaining normal arterial blood pressure and PaCO_2 , and with a $\text{PaO}_2 > 100\text{ mmHg}$. The significance of this study is that fentanyl was shown to affect catecholamine metabolism in the striatum which may relate to rigidity caused by this opiate. Further studies are necessary with voltammetry in the striatum of chronically implanted freely moving rats¹⁶ to show the effect of fentanyl on dopamine metabolism as well as on motor tone.

Acknowledgements

The authors express thanks to Dinah Weissmann for aid in histological assessment, to Annick Barbu for preparation of the manuscript, and to Jean-Yves Gillon for his consultation.

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Résumé

Le mécanisme proposé pour expliquer la rigidité musculaire induite par le fentanyl est celui de l'effet des opiacés sur la transmission dopaminergique dans le corps strié. Le but de cette étude est d'observer les effets du fentanyl sur le courant d'oxydation des catécholamines dans le corps strié des rats (CA.OC) mesuré par voltamétrie in vivo qui reflète la concentration extracellulaire DOPAC (3-4, acide dihydroxy-phenylactique). Des rats mâles Sprague-Dawley furent anesthésiés avec de l'halothane et des électrodes en fibre de carbone ont été implantées dans le corps strié. Après une période de stabilisation initiale d'une heure, les rats ont reçu une injection intraveineuse de salin suivie 30 minutes plus tard par du fentanyl $10 \mu\text{g} \cdot \text{kg}^{-1}$ pour dix minutes et à la soixante-dixième minute ils ont reçu un inhibiteur de la monoamine oxydase, la pargyline $70 \text{ mg} \cdot \text{kg}^{-1}$. Le fentanyl a produit une augmentation significative ($P < 0.05$ Anova) du CA.OC chez tous les animaux. Ceci a atteint un plateau 15 minutes après l'administration de fentanyl pour atteindre un maximum de 148 ± 10.2 pour cent du contrôle 35 minutes après l'administration du fentanyl. La pargyline a produit une chute rapide du CA.OC qui baissa de 143 ± 11.6 à 39 ± 6.8 pour cent du contrôle pour 30 minutes. Il n'y avait aucune différence significative entre le pH, PaO_2 et PaCO_2 lors de l'administration de physiologique et du fentanyl ainsi qu'il n'y avait aucune variation significative de la pression artérielle à travers l'expérience. Cette étude montre que lors des conditions physiologiques stables, le fentanyl produit une augmentation significative du CA.OC dans le corps strié des rats.