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MECHANISMS OF HEAT STRESS POTENTIATION OF FENTANYL LETHALITY IN RATS

Jury Ju. Ivnitsky✉, Ekaterina O. Demydova, Olga A. Vakunenkova, Ekaterina A. Zolotoverkhaja, Alexandr I. Golovko

Golikov Research Center of Toxicology, St. Petersburg, Russia

Introduction. As a rule, preclinical safety assessment of narcotic analgesic agents is conducted at ambient temperatures of 20–24 °C. However, their clinical use may occur under different thermal conditions. Previous studies have demonstrated the ability of heat stress to potentiate the lethal and narcotic effects of fentanyl in rats, yet the mechanisms underlying this phenomenon remain unknown.
Objective. Testing hypotheses on the mechanisms of fentanyl toxicity potentiation in rats under heat stress conditions.
Materials and methods. The study was conducted on outbred male albino rats weighing 191–210 g. We investigated the effects of intravenous fentanyl administration at a dose of 200 μg/kg and/or a 40-min exposure to an ambient temperature of 40 °C on body temperature and mass, brain moisture content and mass, glutamine concentration in brain tissue, biochemical parameters of blood collected from the *a. carotis communis* and *v. jugularis interna*, and oxygen consumption by brain homogenates. Statistical analysis was performed using the OriginPro software.
Results. Fentanyl administration induced opisthotonus, coma, bradypnea, and fundal cyanosis. The 40-min lethality following fentanyl injection was 0–9% at an ambient temperature of 22 °C and 68–71% at an ambient temperature of 40 °C. In surviving rats removed from the thermal chamber, rectal temperature was elevated to 42.9 °C. The relative mass of the freshly isolated brain and the brain dried to a constant weight increased by 7.4% and 7.2%, respectively. Glutamine content in brain tissue increased by 46%. Plasma concentrations of ammonia, creatinine, and lactate were elevated by 2.0–2.2, 2.1–2.3, and 1.5–1.6 times, respectively. In the absence of fentanyl administration, no lethality was observed in rats placed in the thermal chamber. Rectal temperature increased to 42.7 °C. The relative mass of the freshly isolated brain and the dried brain increased by 6.1% and 8.9%, respectively. Brain glutamine content increased by 43%. Plasma creatinine levels rose by 2.2–2.4 times, and lactate levels increased by 25–45%. In the absence of heat stress, fentanyl increased plasma creatinine concentration only by 1.6–1.8 times. The arteriovenous gradient of plasma ammonia concentration was positive in all animals. Oxygen consumption by brain homogenates decreased by 10% under isolated heat stress and increased by 7% under a combined action of heat stress and fentanyl administration.
Conclusions. Hypoxemia, lactic acidemia, and hyperammonemia were necessary conditions for the aggravating effect of heat stress on acute fentanyl intoxication in rats. Conversely, irreversible thermal damage to biological tissues, organism dehydration, cerebral edema, swelling, hyperemia, and glutamine accumulation in the brain were not identified as such necessary conditions.

Keywords: hyperammonemia; brain tissue glutamine; lactic acidemia; lethality; acute intoxication; body temperature; heat stress; fentanyl

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✉ Jury Ju. Ivnitsky neugierig@mail.ru

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МЕХАНИЗМЫ ПОТЕНЦИРОВАНИЯ ТЕПЛОВЫМ СТРЕССОМ ЛЕТАЛЬНОГО ДЕЙСТВИЯ ФЕНТАНИЛА НА КРЫС

Ю.Ю. Ивницкий✉, Е.О. Демидова, О.А. Вакуненкова, Е.А. Золотоверхая, А.И. Головко

Научно-клинический центр токсикологии им. академика С.Н. Голикова Федерального медико-биологического агентства, Санкт-Петербург, Россия

Введение. Доклиническое изучение безопасности наркотических анальгетиков проводят при температуре воздуха 20–24 °С, но их применение возможно и при иной температуре. Ранее показано потенцирование тепловым стрессом летального и наркотического действия фентанила на крыс, однако механизмы этого феномена неизвестны.
Цель. Проверка гипотез о механизмах повышения токсичности фентанила для крыс в условиях теплового стресса.
Материалы и методы. Исследование проведено на беспородных самцах крыс-альбиносов массой 191–210 г. Изучали влияние внутривенного введения фентанила в дозе 200 мкг/кг и (или) сорокаминутного пребывания при температуре воздуха 40 °С на температуру и массу тела, влагосодержание и массу головного мозга, содержание глутамина в ткани мозга, биохимические показатели крови из *a. carotis communis* и *v. jugularis interna* крыс, потребление кислорода гомогенатами их головного мозга. Статистический анализ проводили с использованием программного обеспечения OriginPro.
Результаты. Введение фентанила вызывало опистотонус, кому, брадипноэ и цианоз глазного дна. Летальность за 40 мин после введения фентанила составляла 0–9% при температуре воздуха 22 °С и 68–71% при температуре воздуха 40 °С. Ректальная температура у выживших крыс по извлечении из термокамеры была повышена до 42,9 °С, относительная масса свежеизвлеченного

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и высушенного до постоянной массы головного мозга — на 7,4 и 7,2% соответственно, содержание глутамина в его ткани — на 46%; в плазме крови повышалась концентрация аммиака в 2,0–2,2 раза, креатинина — в 2,1–2,3 раза и лактата — в 1,5–1,6 раза. Без введения фентанила летальность крыс в термокамере отсутствовала, ректальная температура повышалась до 42,7 °С, относительная масса свежеизвлеченного головного мозга — на 6,1% и сухого — на 8,9%, содержание в нем глутамина — на 43%, уровень в плазме крови креатинина — в 2,2–2,4 раза и лактата — на 25–45%. Без теплового стресса фентанил лишь повышал в 1,6–1,8 раза концентрацию креатинина в плазме крови. Артериовенозный градиент концентрации аммиака в плазме крови у всех животных был положительным. Потребление кислорода гомогенатами головного мозга снижалось на 10% при изолированном тепловом стрессе и повышалось на 7% при тепловом стрессе на фоне введения фентанила.

Выводы. Гипоксемия, лактацидемия и гипераммониемия были необходимыми условиями отягощающего влияния теплового стресса на острую интоксикацию фентанилом у крыс. Необратимое термическое повреждение биотканей, обезвоживание организма, отек, набухание, гиперемия головного мозга или накопление в нем глутамина не являлись такими условиями.

Ключевые слова: гипераммониемия; глутамин ткани мозга; лактацидемия; летальность; острая интоксикация; температура тела; тепловой стресс; фентанил

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Финансирование: исследование выполнено без спонсорской поддержки.

Соответствие принципам этики: исследование выполнено с соблюдением правил биоэтики, утвержденных Европейской конвенцией о защите позвоночных животных, используемых для экспериментальных и других целей. Проведение исследований одобрено на заседании биоэтического комитета ФГБУ НКЦТ им. С.Н. Голикова ФМБА России (протокол № 16/24 от 22.10.2024).

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✉ Ивницкий Юрий Юрьевич neugierig@mail.ru

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INTRODUCTION

As a rule, preclinical drug studies are conducted at an ambient temperature of 20–24 °C, as stipulated by the Guidelines for the Housing and Care of Laboratory Animals.[1] Consequently, the influence of heat stress on the biological activity of a number of pharmaceuticals is poorly represented in the scientific literature, despite its potential role as a background condition in real-world use, particularly in prehospital settings. This underscores the relevance of investigating the biological activity of drugs under climatic conditions that promote body hyperthermia.

We have previously demonstrated [1] that intravenous administration of fentanyl to rats at doses not exceeding the LD_5 (at an ambient temperature of 20–24 °C) becomes uniformly lethal upon subsequent 40-min exposure to an ambient temperature of 40 °C, a condition that is well-tolerated in the absence of fentanyl. In order to explain this previously unknown phenomenon, hypotheses suggesting hyperammonemia and the resulting brain swelling as mechanisms for the aggravating effect of heat stress on acute fentanyl intoxication were advanced.

The aim of this study was to test the above hypotheses regarding the mechanisms of fentanyl toxicity enhancement in rats under heat stress conditions.

MATERIALS AND METHODS

The study was conducted from January to May 2025 on outbred male albino rats (191–210 g) obtained from the Rappolovo Laboratory Animal Nursery (Kurchatov Institute). Two experimental series were performed.

In the first series of experiments (*n* = 77), we investigated the effects of isolated or combined exposure to fentanyl and heat stress on rectal temperature, body and brain mass, brain glutamine content, and biochemical parameters of blood collected from the *a. carotis communis* and *v. jugularis interna*. The study design and animal distribution into experimental groups are presented in Table 1.

In the second series of experiments, a separate cohort of rats (*n* = 37), comparable to those used in the first series, was employed to investigate the effects of isolated or combined exposure to fentanyl and heat stress on oxygen consumption by brain homogenates. The study design is presented in Table 2.

A fentanyl solution of 50 μg/mL (Moscow Endocrine Plant, batch 30212) was administered to the animals at a volume of 4 mL/kg via the lateral tail vein. The fentanyl dose was 200 μg/kg, which, according to previous data [1], corresponded to $LD_{5/24\,h}$ at 22 °C and $LD_{70/24\,h}$ at 40 °C over 40 min, followed by maintenance at 22 °C.

Thermal stress was simulated in a BMT Stericell SC 111 ECO thermal chamber (Czech Republic) with a volume of 111 L. The following environmental parameters were maintained: air temperature at 40 ± 1 °C, relative humidity at 48%, and an air exchange rate of 45 chamber volumes per hour. During their stay in the thermal chamber, all animals were placed in restrainers made of perforated aluminum. Rats that did not receive fentanyl and were kept in restrainers at room temperature were considered intact (control) animals.

[1] GOST 33216-2014 “Guidelines for the Housing and Care of Laboratory Animals. Requirements for the Maintenance and Care of Laboratory Rodents and Rabbits”.

Table 1. Protocol for the assessment of fentanyl and/or heat stress effects on body temperature, body and brain mass, glutamine content in dry brain tissue, and biochemical parameters of blood from the common carotid artery and internal jugular vein in rats

Number of rats surviving until examination	Exposure of rats		Parameters
	Pharmacological	Microclimatic	
11 out of11	absent	22 °C for 40 min post-injection	body mass and temperature, brain mass, glutamine content in dry brain tissue, and levels of ammonia, creatinine, and lactic acid in blood from the *a. carotis communis* and *v. jugularis interna* at 40 min post-injection
10 out of 11	fentanyl		
10 out of 10	absent	40 °C for 40 min post-injection	
13 out of 45	fentanyl		

Table compiled by the authors

Table 2. Protocol for the assessment of fentanyl and/or heat stress effects on oxygen consumption by rat brain homogenates

Number of rats surviving until examination	Exposure of rats		Parameters
	Pharmacological	Microclimatic	
6 out of 6	absent	22 °C for 40 min post-injection	oxygen consumption by brain homogenates extracted 40 min after injection
6 out of 6	fentanyl		
6 out of 6	absent	40 °C for 40 min post-injection	
6 out of 19	fentanyl		

Table compiled by the authors

Body temperature was measured with a precision of 0.1 °C using an electric thermometer with a rat-specific probe (RET-2, WPI, China). The probe tip was inserted 3 cm into the rectum. Body weight was measured with a precision of 1g both before and 40 min after administration of the solutions to the animals. The color of the ocular fundus was assessed indirectly by observing the eye color in albino rats, which lack pigments other than hemoglobin.

In the first series of experiments, rats were immobilized in the dorsal position for blood collection; those not receiving fentanyl were subjected to light halothane anesthesia. Blood samples of 0.5–1 mL were collected from the *a. carotis communis* and *v. jugularis interna*. Plasma was separated by centrifugation at 4 °C for 10 min at 3000 rpm (5400 g). Using an A-25 biochemical analyzer (BioSystems, Spain), the following parameters were measured:

- Ammonia — using an Ammonia Ultra reagent kit (Sentinel Diagnostics, Italy);
- Creatinine — via the reaction with picric acid, using a Creatinine-Novo-A reagent kit (Vector-Best, Russia);
- Lactic acid — using a Lactate Vital reagent kit (Vital Development Corporation, Russia).

Following the completion of blood collection, the animals were decapitated; the brain was removed and weighed to the nearest 0.01 g both before and after drying to constant weight at 105 °C. The dried brain was ground to a powder, suspended in nine parts of 0.01 M phosphate buffered saline (pH = 7.0), mixed, and centrifuged at 20,000 rpm (36,000 g) for 1 h at 4 °C. The supernatant was incubated for 10 min at 100 °C in the presence of sulfuric acid at a final concentration of 0.17 M. Ammonia was measured in the supernatant both before and after incubation using the method described above. Glutamine concentration in the tissue extract was determined as the difference between ammonia concentrations in the supernatant before and after acid hydrolysis [2]. An L-glutamine standard (Merck, Germany) was used to construct the calibration curve. Glutamine content in dry tissue was calculated in µmol/g.

In the second series of experiments, the brain was removed, weighed, and homogenized in cold Hank's solution (free of phenol red) to produce a uniform suspension, with a ratio of one mass unit of wet tissue to four mass units of Hank's solution. One milliliter of the resulting 20% homogenate was added to manometric vessels containing 2 mL of Hank's solution (without phenol red) in the main chambers and 0.2 mL of 10% potassium hydroxide solution in the central compartments.

Oxygen consumption was measured using the manometric method [3] with a Warburg apparatus (model Wa 0110, Glaswerke Ilmenau, GDR) at 38 °C and a shaker speed of 120 min^{-1}; air served as the gas phase.

Manometer readings were recorded after a 10-min temperature equilibration period, then at 10-min intervals over a 70-min span. Oxygen consumption was expressed in microliters per gram of wet tissue, cumulatively.

Statistical analysis was performed using the OriginPro software. Results were presented as the mean value and its standard error ($M \pm m$). To test the normality of the distribution, the Shapiro–Wilk test was used. The effect of the applied interventions on parametric parameters was assessed using analysis of variance (ANOVA). When the obtained models were statistically significant, post-hoc comparisons of mean values between groups were conducted using Tukey's honest significant difference (HSD) test. The critical significance level α was set at 0.05.

RESULTS

Within several seconds after fentanyl administration, rats developed opisthotonus, tail extension, brief apnea followed by shallow, infrequent breathing, and pronounced cyanosis of the ocular fundus. The absence of auditory-motor and pain reflexes allowed the animals' neurological status to be classified as coma.

In the first series of experiments, the mortality rate among rats that received fentanyl over a 40-min period at 22 °C was 9%, while at 40 °C it reached 71%. For rats that did not receive fentanyl, a 40-min exposure to 40 °C was non-lethal. Thus, upon removal from the thermal chamber, their behavior appeared normal, and the ocular fundus exhibited a bright crimson color.

In surviving rats, the baseline rectal temperature was 38.0 ± 0.1 °C ($n = 44$). In total, 40 min after fentanyl administration, the rectal temperature of animals maintained at 22 °C decreased to 33.9 ± 0.2 °C ($n = 10$), while that of animals placed in the thermal chamber increased to 42.9 ± 0.3 °C ($n = 12$). Exposure to the thermal chamber without fentanyl administration increased rectal temperature to 42.7 ± 0.3 °C ($n = 10$). These changes are presented in Fig. 1A.

Within 40 min after fentanyl injection, the body mass of rats kept at 22 °C decreased from 203 ± 5 g to 198 ± 5 g, with a similar trend observed in intact animals. Exposure to the thermal chamber without fentanyl administration reduced body mass from 200 ± 4 g to 190 ± 4 g, while in survivors receiving fentanyl, it decreased from 201 ± 5 g to 192 ± 5 g. Changes in body mass are shown in Fig. 1B.

The absolute mass of the isolated brain, as well as its ratio to the initial body mass of rats that received fentanyl and/or underwent heat stress, did not differ significantly from those in intact animals. The brain-to-body mass ratio 40 min after fentanyl administration and/or placement in the thermal chamber showed a tendency to increase in all groups compared to the intact group. For animals placed in the thermal chamber after fentanyl administration, this tendency was significant and manifested as a 7.4% increase in their relative brain mass compared to that in intact rats (Fig. 2A).

In intact rats, brain moisture content averaged 79.4%. Under isolated fentanyl exposure, it was 79.2%; under isolated hyperthermia, 78.9%; and under combined hyperthermia and fentanyl exposure, 79.5%. The intergroup differences were not statistically significant. The relative mass of the dry brain following exposure to fentanyl and heat stress exceeded that in intact rats by 7.2%, and after isolated heat exposure, by 8.9% (Fig. 2B).

In the extract of dry brain tissue, the concentration of free ammonia was below the detection limit of the method (15 μmol/L). However, following acid hydrolysis, it was detected at a level equivalent to the glutamine content. In intact rats, the glutamine concentration in dry brain tissue was 21.8 ± 1.5 μmol/g, which corresponded to 4.5 μmol/g in wet tissue and was consistent with the data published in [4]. In dry brain tissue subjected to isolated

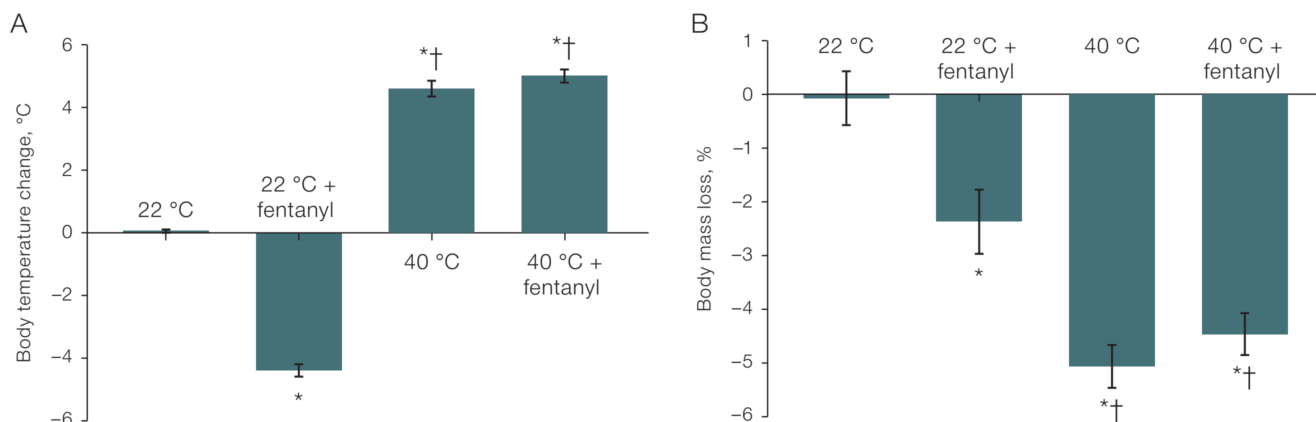

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Fig. 1. Changes in rectal temperature and body mass of rats following administration of fentanyl at a dose of 200 μg/kg and/or a 40-min exposure to an ambient temperature of 22 °C or 40 °C

Note: the results are presented as $M \pm m$; statistically significant difference $p < 0.05$: * — with intact rats kept for 40 min at room temperature; † — with rats that received fentanyl and were kept for 40 min at room temperature.

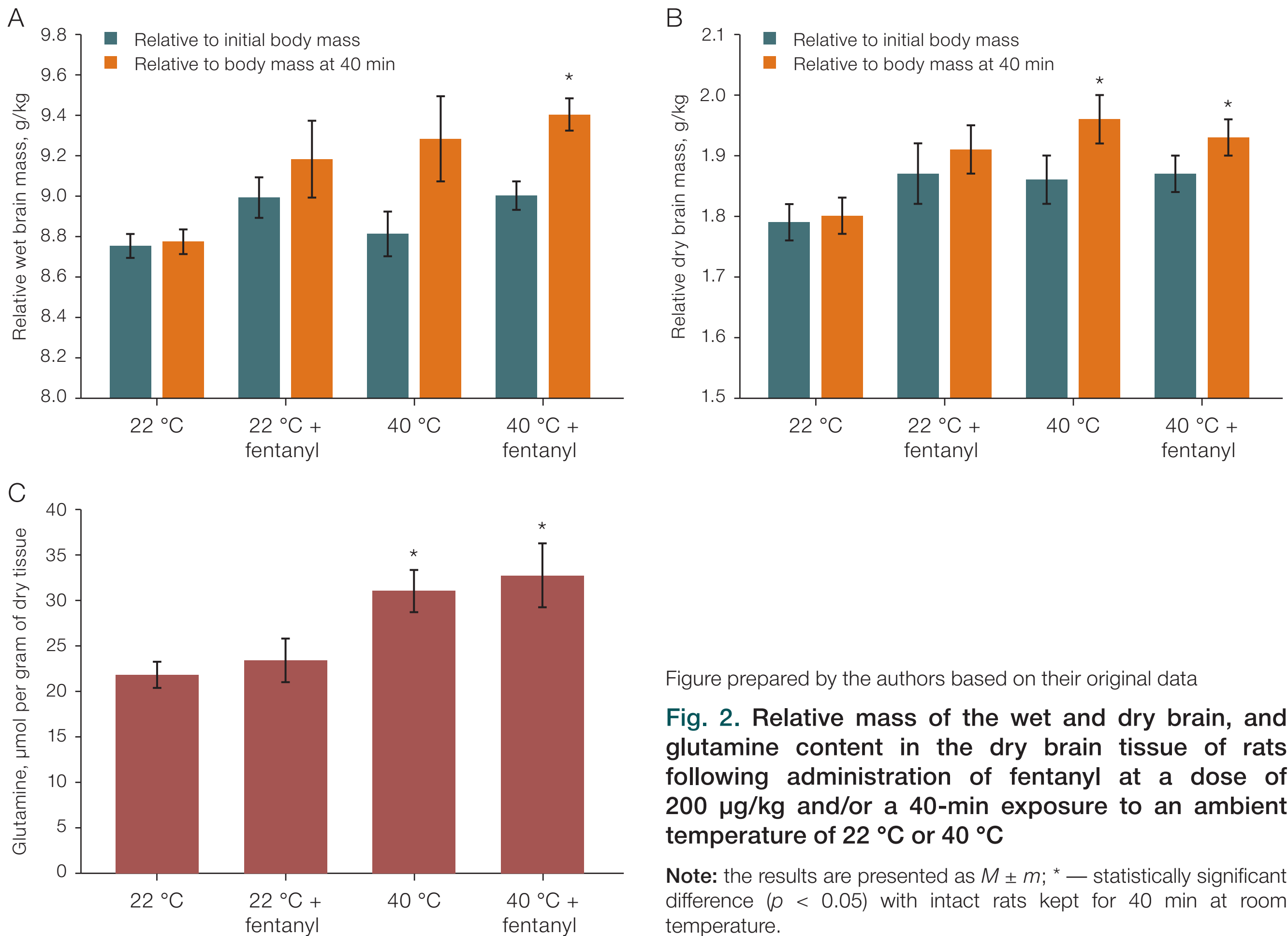


Figure prepared by the authors based on their original data

Fig. 2. Relative mass of the wet and dry brain, and glutamine content in the dry brain tissue of rats following administration of fentanyl at a dose of 200 μg/kg and/or a 40-min exposure to an ambient temperature of 22 °C or 40 °C

Note: the results are presented as $M \pm m$; * — statistically significant difference ($p < 0.05$) with intact rats kept for 40 min at room temperature.

heat stress or its combination with fentanyl exposure, the glutamine content was increased by 43% and 46%, respectively, compared to intact animals (Fig. 2C).

In the setting of combined fentanyl and heat stress exposure, the blood ammonia level was elevated by 2–2.2 times compared to intact animals. The value of the arteriovenous gradient of plasma ammonia concentration was positive in all animals. Under isolated heat stress, its value of 47 ± 13 μM was equal to that in intact rats, while under isolated fentanyl exposure or in combination with hyperthermia, it showed a tendency to increase: 87 ± 29 and 61 ± 18 μM, respectively (Fig. 3A).

The concentration of creatinine in the plasma of arterial and venous blood exceeded that in intact rats by 1.6–2.4 times in all experimental groups. This increase was the greatest (2.2–2.4-fold) under conditions of heat stress (Fig. 3B).

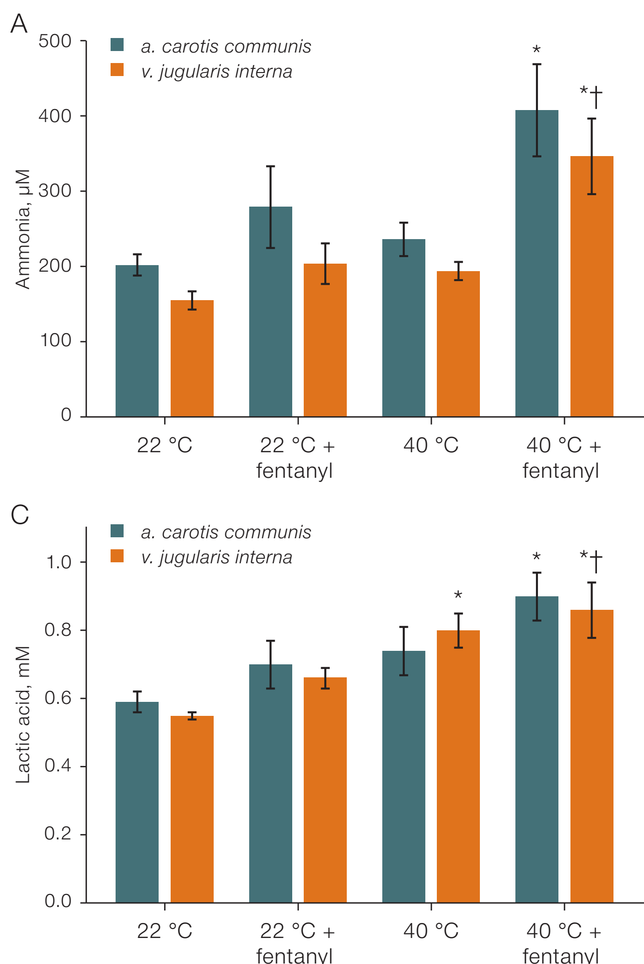
In the setting of heat stress, the level of lactic acid in the plasma of arterial and venous blood was 25–45% higher than in intact rats, and under hyperthermia combined with fentanyl administration, it was 50–60% higher. The lactate content in arterial blood exceeded its concentration in venous blood in all animals except those subjected to isolated heat stress (Fig. 3C).

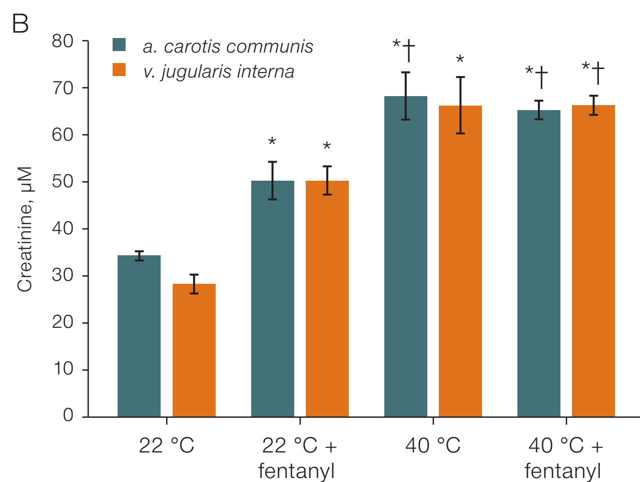
In the second series of experiments, isolated exposure to fentanyl or heat stress was non-lethal, while their combination resulted in the death of 13 (68%) out of 19 rats before removal from the thermal chamber.

The respiratory rate of brain homogenates remained stable across all experimental groups, ensuring a linear dynamic of oxygen consumption volume. For intact animals, it was described by the linear regression equation $y = 3.83\,x$; for isolated fentanyl exposure — $y = 3.71\,x$; for isolated heat stress — $y = 3.41\,x$; and for the combined action of these agents — $y = 4.09\,x$, where x is the incubation duration (min) and y is the specific volume of oxygen consumed (μL per 1 g of tissue). The mean oxygen consumption over 70 min was 3.8 μL/(g × min) in the intact group. Under isolated heat stress, it was 10% lower, and under heat stress combined with fentanyl action, it was 7% higher than in intact rats (Fig. 4).

DISCUSSION

In the thermal chamber, the rectal temperature of the rats increased, indicating the development of heat stress. Contrary to the hypothermic effect of fentanyl observed in the present study and described in the literature [5], after 40 min in the thermal chamber, the body temperature of rats that received fentanyl, as well as those under isolated heat exposure, approached 43 °C. This is known to be a threshold for irreversible tissue damage





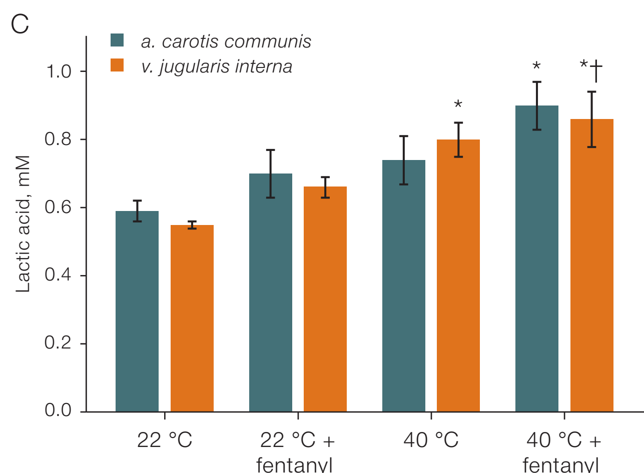


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Fig. 3. The levels of ammonia, creatinine, and lactic acid in blood plasma from the common carotid artery and internal jugular vein of rats following administration of fentanyl at a dose of 200 μg/kg and/or 40-min exposure to air temperatures of 22 °C or 40 °C

Note: the results are presented as $M \pm m$; statistically significant difference, $p < 0.05$: * — with intact rats kept for 40 min at 22 °C (and, for ammonia, also at 40 °C); † — with rats treated with fentanyl and were kept for 40 min at 22 °C.

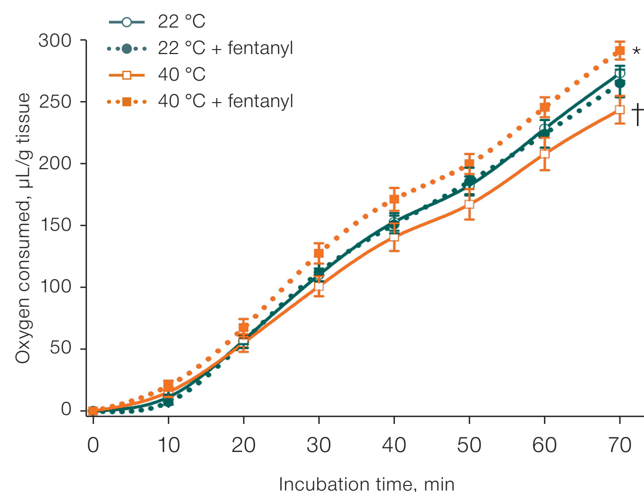

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Fig. 4. Oxygen consumption by brain homogenates following administration of fentanyl at a dose of 200 μg/kg to rats and/or their 40-min exposure to an ambient temperature of 22 °C or 40 °C

Note: the results are presented as $M \pm m$; statistically significant difference, $p < 0.05$: * — with intact rats left at room temperature for 40 min; † — rats treated with fentanyl and left for 40 min at an air temperature of 40 °C.

[6]. However, lethality was observed only in the setting of fentanyl administration, and, therefore, it was not caused by thermal damage to tissues or by a decrease in hemoglobin affinity for oxygen with increasing body temperature. For the same reason, lethality was not determined by organism dehydration: both water loss, leading to a decrease in body mass, and hemoconcentration, manifested by the accumulation of creatinine in the blood, were comparable in animals that received and did not receive fentanyl. The increase in blood viscosity due to dehydration could impede blood supply to the brain; however, in the absence of fentanyl, it was not lethal.

The hypothesis suggesting the contribution of brain swelling to the aggravating effect of heat stress on acute fentanyl intoxication was not experimentally confirmed. In rats that survived 40 min after the onset of the combined exposure to these factors, the increase in the relative mass of the wet and dry brain was comparable (by 7.4% and 7.2%, respectively), and the moisture content did not change significantly. This indicates the absence of pronounced cerebral edema, swelling, or hyperemia in these animals.

Under heat stress, brain dehydration was less pronounced than systemic dehydration, as evidenced by an increase in its relative mass while the absolute mass remained stable. Given that the mass of biological tissue

is positively correlated with its volume, the ratio of brain volume to cranial cavity volume during heat exposure could have increased, potentially elevating intracranial pressure. In accordance with Pascal's law, this excess hydraulic pressure, if transmitted to blood vessels, would impede cerebral blood flow. However, the comparable extent of increased relative brain mass in rats that did and did not receive fentanyl prior to placement in the thermal chamber — coupled with survival only in the latter group — does not support elevated intracranial pressure as a key mechanism in thanatogenesis.

Under the influence of fentanyl and/or heat stress, glutamine accumulated in the brain. Calculated per 1 g of wet tissue, its mean group content was higher than in intact rats by 0.2, 1.0, and 1.0 μmol at 40 min after fentanyl administration, placement in the thermal chamber, or a combination of these factors, respectively. Since postmortem synthesis of glutamine in brain tissue is impossible due to the cessation of oxidative phosphorylation within 10.5 s after decapitation [7], this increase reflected the direction of in-vivo changes in the primary reaction of ammonia detoxification by the brain: the synthesis of glutamine by astrocytic glutamine synthetase [8]. The comparable extent of glutamine accumulation in the brains of rats that did and did not receive fentanyl prior to placement in the thermal chamber allows this disturbance to be excluded from being a lethal factor.

A positive arteriovenous transcerebral gradient of plasma ammonia concentration was observed in all rats, which is consistent with the data reported in [8] and indicates that the primary source of glutamine in brain tissue was ammonia extracted from the blood. Each milliliter of blood from intact animals or those subjected to isolated heat stress lost 47 nmol of ammonia during its passage from the common carotid artery to the internal jugular vein. Under isolated fentanyl exposure, this loss amounted to 87 nmol, and under combined fentanyl and heat stress, it was 61 nmol. The volumetric rate of cerebral blood flow in rats is estimated at 1.3 mL/(g × min) [9]. Assuming unchanged cerebral blood flow, this allows for the calculation of the amount of ammonia absorbed by the tissues of the head (predominantly the brain) from the blood: 2.4, 4.5, 2.4, and 3.2 μmol per 1 g of tissue over 40 min for intact animals, those receiving fentanyl, those subjected to heat stress, or the combined action of both factors, respectively. However, these data do not reveal a consistent correlation with the intergroup differences in lethality.

The increase in glutamine content in the brain tissue did not account for the binding of all the ammonia absorbed from the blood. The remaining portion of ammonia, which crossed the blood–brain barrier over 40 min, can be determined by subtracting the increase in tissue glutamine content from the amount of ammonia absorbed by the tissue. This value amounts to 2.4, 4.3, 1.4, and 2.2 μmol per 1 g of tissue for intact animals, those receiving fentanyl, those subjected to heat stress, or the combined action of both factors, respectively. In neurons, ammonia is stoichiometrically incorporated into glutamate and other amino acids. Since the calculated amounts of unconsumed ammonia are an order of magnitude higher than the content of pyruvic (0.06–0.13 μmol/g) and α-ketoglutaric (0.12–0.19 μmol/g) oxoacids in the nervous tissue [10], their amination to form alanine and glutamate could have reduced the availability of these key intermediates of energy metabolism for the brain tissue.

The availability of pyruvate could also have been reduced by its carboxylation to form oxaloacetate. This process is stimulated by low oxaloacetate concentration, which is linked to the concentration of α-ketoglutarate; an excess of ammonia in tissues leads to the amination of precisely these keto acids. The depleting effect of ammonia on the pool of Krebs cycle intermediates in neurons, which triggers a cascade of neuronal damage, is considered by some authors to be a primary mechanism of its neurotoxicity [11]. Another potential mechanism of neurotoxicity for ammonia not incorporated into glutamine could be the accumulation of the excitotoxic agent, glutamate, in the brain [12]. As evident from the data obtained, the intergroup differences in the calculated amounts of ammonia involved in these processes could not explain the potentiation of the acute toxicity of fentanyl under the action of heat stress.

A 2.0–2.2-fold increase in blood ammonia levels, which distinguished rats after combined exposure to fentanyl and heat stress from those subjected to isolated exposure, is generally considered non-lethal [8]. However, it can be hypothesized that in animals that died before removal from the thermal chamber, hyperammonemia was more severe than in those that did survive. Furthermore, hyperammonemia was accompanied by hypoxemia, indicated by fundal cyanosis, and metabolic acidosis, marked by lactic acidemia. The presence of both these disturbances and the maximal severity of lactic acidemia distinguished animals subjected to the combined action of fentanyl and heat stress from those subjected to their isolated effects. Hypoxia and metabolic acidosis are known to increase the toxicity of ammonia [13, 14]. Thus, it is evident that hyperammonemia, in combination with hypoxemia and lactic acidemia, reduced the survival of rats under the conditions of fentanyl and heat stress exposure.

Oxygen consumption by aerobic cells is an integral indicator of their metabolic activity [15]. Oxygen consumption by brain cells was measured in vitro under the conditions free from substrate limitations and with the ATP-dependent function of nerve impulse generation and conduction suppressed. It ranged 3.4–4.1 μL per 1 g of tissue per minute, which is an order of magnitude lower than the in-vivo rate of 33 μL per 1 g of tissue per minute [7]. The data obtained did not reveal any impairments in cellular respiration in rats that survived exposure to fentanyl and heat stress that would prevent the meeting of the energy demands of the nervous tissue under these favorable in-vitro conditions. The observed intensification of oxygen consumption by brain

homogenates from these animals was compensatory in nature and reflected a state of energy deprivation in which the brain tissue existed prior to decapitation. Given the presence of lactic acidemia and signs of hypoxemia in these animals, positive modulators of cellular respiration — ADP, AMP, and phosphate — were likely accumulating in their brain tissue [16].

Since the tissue was diluted 15-fold during homogenate preparation, the stimulatory effect of these substances was manifested only in a weakened form. Under isolated fentanyl exposure, the aforementioned disturbances might have been mitigated by a reduced demand for ATP in the brain due to the comatose state [17], which is a probable reason for the absence of significant changes in oxygen consumption by the brain homogenates. The lack of substantial changes in blood lactate levels and its arteriovenous gradient in these animals indicated an absence of significant energy debt in their brain tissue. In rats subjected to isolated heat stress, the bright scarlet color of the ocular fundus suggested intense cerebral blood flow and high oxygen saturation of arterial hemoglobin. In these animals, the lower oxygen consumption by brain cells in vitro compared to intact rats might reflect the accumulation of a negative modulator of cellular respiration — ATP.

The data obtained demonstrate that a triad of metabolic disturbances — lactic acidemia, hypoxemia, and hyperammonemia — mediates the aggravation of acute fentanyl intoxication by heat stress in rats. While the emergence of the former two components of this triad during hyperthermia and impaired external respiration is expected, the mechanisms underlying hyperammonemia remain to be identified. In light of the cellular stress concept [18] and considering its impact on intestinal permeability [19], it is pertinent to evaluate the role of the gut barrier in the development of hyperammonemia during body hyperthermia in the setting of acute fentanyl intoxication.

CONCLUSION

1. Simulating microclimatic conditions that induce nonlethal acute body hyperthermia increased the toxicity of fentanyl in rats, raising the 40-min lethality to 68–71% compared to 0–9% at room temperature.
2. The potentiation of the lethal effect of fentanyl by heat stress in rats was not caused by irreversible thermal damage to biological tissues, organism dehydration, cerebral edema, swelling, hyperemia, or glutamine accumulation in the brain.
3. Hypoxemia, lactic acidemia, and hyperammonemia were necessary conditions for the aggravating effect of conditions promoting body hyperthermia on acute fentanyl intoxication in rats.

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Authors:

Jury Ju. Ivnitsky, Dr. Sci. (Med.), Professor, ORCID: https://orcid.org/0000-0002-1057-5356
Ekaterina O. Demydova, ORCID: https://orcid.org/0009-0003-0820-8471
Olga A. Vakunenkova, ORCID: https://orcid.org/0009-0005-9665-9866
Ekaterina A. Zolotoverkhaja, Cand. Sci. (Biol.), ORCID: https://orcid.org/0000-0002-9708-2596
Alexandr I. Golovko, Dr. Sci. (Med.), Professor, ORCID: https://orcid.org/0000-0002-2751-3637