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# Paracetamol-Induced Hypothermia in Rodents: A Review on Pharmacodynamics

**Laurențiu Coman [1,†], Horia Păunescu [2,*], Cristina Isabel Viorica Ghiță [2,†], Radu Ciprian Țincu [3,4,†], Sorina Vasile [5], Delia Cinteza [6,7], Ion Fulga [2] and Oana Andreia Coman [2]**

1 Department of Physiology, Faculty of Pharmacy, "Carol Davila" University of Medicine and Pharmacy, 020021 Bucharest, Romania; laurentiu.coman@umfcd.ro
2 Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, "Carol Davila" University of Medicine and Pharmacy, 020021 Bucharest, Romania; isabel.ghita@umfcd.ro (C.I.V.G.); ion.fulga@umfcd.ro (I.F.); oana.coman@umfcd.ro (O.A.C.)
3 Department of Clinical Toxicology, Faculty of Medicine, "Carol Davila" University of Medicine and Pharmacy, 020021 Bucharest, Romania; radu.tincu@umfcd.ro
4 Critical Care Toxicology Department, Bucharest Clinical Emergency Hospital, 020021 Bucharest, Romania
5 Infectious Diseases Resident Physician, Guiding Hospital: "Victor Babeș" Hospital of Infectious and Tropical Diseases, 030303 Bucharest, Romania; sorina-marinela.vasile@rez.umfcd.ro
6 Department of Rehabilitation and Physical Medicine, Faculty of Medicine, Carol Davila University of Medicine and Pharmacy, 030079 Bucharest, Romania; delia.cinteza@umfcd.ro
7 National Institute of Rehabilitation, Physical Medicine and Balneoclimatology, 030079 Bucharest, Romania
* Correspondence: horia.paunescu@umfcd.ro; Tel.: +4-0723-867-286
† These authors contributed equally to this work.

**Abstract:** Paracetamol can induce hypothermia in humans and rodents. The study's aim is to review the mechanisms of paracetamol-induced hypothermia in rodents or the results issued from in vitro studies on the same species' tissues (in doses that do not produce hepatic impairment) using the latest developments published in scientific journals over the last 15 years. Available human studies are also analysed. An extensive search in PubMed databases exploring the hypothermic response to paracetamol was conducted. 4669 articles about paracetamol's effects on body temperature in mice or rats were found. After applying additional filters, 20 articles were selected for review, with 9 of them presented in tabular forms. The analysis of these articles found that the hypothermic effect of paracetamol is due to the inhibition of a cyclooxygenase-1 variant, is potentiated by endothelin receptor antagonists, and can be mediated through $GABA_A$ receptors and possibly through transient receptor potential cation channel subfamily A member 1 via N-acetyl-p-benzoquinone imine in the central nervous system. Human studies confirm the in vivo and in vitro experiments in rodents regarding the presence of a hypothermic effect after high, non-toxic doses of paracetamol. Further research is required to understand the mechanisms behind paracetamol's hypothermic effect in humans.

**Keywords:** paracetamol; hypothermia; rodents; mechanism of action

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## 1. Introduction

Paracetamol (acetaminophen) is one of the most commonly prescribed drugs worldwide. Synthetized over 150 years ago, paracetamol is highly efficient as analgesic and antipyretic and is on the list of the World Health Organization's essential medicines. However, the drug is considered to be devoid of anti-inflammatory properties [1].

High doses of paracetamol pose a serious risk for hepatotoxicity due to the lack of neutralisation of the oxidation metabolite N-acetyl-p-benzoquinone imine (NAPQI), secondary to the depletion of glutathione [2].

Aside from the analgesic and antipyretic effects, paracetamol also presents hypothermic effects. Hypothermia induced by paracetamol was firstly described in the 1960s in rats treated with high doses of the drug in the presence of hepatic failure. A decade later,

paracetamol was shown to produce profound hypothermia when administered intracerebroventricularly [3]. In 1982, Massey et al. demonstrated its hypothermic effects in mice treated with non-toxic doses [4].

The serendipitous discovery of the hypothermic effect, together with the clinical implications of the therapeutic induced hypothermia, led to new pharmacodynamic studies to understand the mechanism behind paracetamol's effect of lowering body temperature. A significant number of studies started their investigations using the methodologies previously employed by researchers to determine the mechanisms of the analgesic effects of paracetamol.

It is thus essential to briefly revisit some of the past scientific perspectives on paracetamol's analgesic and antipyretic effects for a better understanding of the state of the art on the hypothermic effect of paracetamol.

### *1.1. Short History of the Research Regarding the Mechanisms of Paracetamol-Induced Analgesic and Antipyretic Effects*

For a long time, the analgesic and antipyretic activities of paracetamol were believed to be explained mainly by the inhibition of cyclooxygenase (COX) at the central nervous system (CNS) level [5].

Moreover, an argument for this theory was the fact that paracetamol acts especially in sites with low amounts of peroxides—like those found in the brain tissues—because of the interference of the drug with the peroxidase site of COX (POX-), which leads to paracetamol inactivation [6]. The high amounts of peroxides at the sites of inflammation can also be an explanation of the fact that paracetamol does not present anti-inflammatory properties [7].

More specifically, the analgesic and antipyretic effects of paracetamol were suggested to be produced by inhibition of the COX-1 and COX-2 subtypes [8]. However, data showed that COX-2 inhibition is less involved in the analgesic effects, as Ayoub et al., 2006, demonstrated that paracetamol also has an analgesic effect in COX-2 knockout (K.O.) mice [9].

Chandrasekaran et al., 2002, revealed that the COX-3 subtype (with one intron-retaining gene of COX-1) was the target molecule for paracetamol's central analgesic mechanism [10], but this mechanism is probably irrelevant in humans and rodents, according to Graham G.G. and Scott K.F., 2005 [11].

In more recent years, the lack of proof that the analgesic effects of paracetamol are dependent only on COX's central inhibition has made researchers discover and describe other peripheral and non-COX effects [12].

Pini et al., 1996, demonstrated that paracetamol's analgesic effects also depend on the serotoninergic system, showing that the depletion of serotonin in mice brains (with D,L p-chlorophenylalanine) prevents the antinociceptive effect of paracetamol [13]. Moreover, the opioid and NMDA glutamatergic mechanisms of action were also researched for explaining paracetamol-induced analgesia [14–16].

### *1.2. Recent Developments on Paracetamol's Analgesic and Antipyretic Effects*

In the last 15 years, there have been numerous studies focused on the role of the cannabinoid system in explaining the analgesic effect of paracetamol. Högestätt E.D. et al. (2005) showed that paracetamol is metabolised in two steps: firstly, to p-aminophenol, and secondly, to N-arachidonoyl-phenol amine (AM404), a derivative of the arachidonic acid [17].

AM404 acts as a TRPV1 agonist (transient receptor potential cation channel subfamily V member 1) and an anandamide reuptake inhibitor, which causes an increase in endogenous cannabinoids. Cannabinoids produce anti-nociceptive effects that are primarily mediated by CB1 receptors [17–19]. Recent studies have also shown that AM404 may have an additional anti-inflammatory role, inhibiting COX activity and prostaglandin synthesis in the CNS [20].

Another line of research that expanded in recent years is the investigation of serotoninergic involvement in paracetamol's analgesic effect. Paracetamol increases serotonin levels, especially in the pons and frontal cortex of rats, further involving both 5-$HT_2$ receptors and opioid receptors ($\mu_1$ and $\kappa$) [21,22]. Moreover, 5-$HT_2$ and 5-$HT_3$ receptors seem to play an important role in the analgesic effect of paracetamol, while the implications of 5-$HT_1$ receptors are still unclear [23,24]. However, it should be noted that there does not seem to be any direct binding of paracetamol with either the 5-$HT_2$ receptors or with the 5-$HT_1$ or 5-$HT_3$ receptors, and the mechanism is likely to be indirect [24–26].

The latest developments on paracetamol's analgesic effect are those that involve blocking the CNS T-type Cav3.2 calcium channels and stimulating the Kv7 potassium channels found in the dorsal root ganglion and spinal dorsal horn neurons. These discoveries may represent new targets in analgesia. The latest data is best summarised in a recent review of Przybyła G.W. et al., 2020 [27].

### *1.3. The Role of Paracetamol in Inducing Hypothermia as a Therapeutic Option*

The hypothermic effect of different substances, including paracetamol, became of clinical interest in the early 2000s when two prospective randomized trials indicated that inducing hypothermia may be beneficial for some cardiac arrest patients [28].

Nowadays, inducing a state of mild hypothermia (34–35 °C) named "targeted temperature management" represents an efficacious therapeutic option for neuroprotection in different neurological injuries secondary to ischemic stroke, post-cardiac arrest, post-traumatic brain injury with high intracranial pressure, or to perinatal asphyxia-related cardiac arrest in newborns [29–33].

However, paracetamol alone did not show any significant benefits when investigated in two trials that studied targeted temperature management. High doses of paracetamol induced small decreases in core body temperature (CBT) in normothermic or subfebrile patients with ischaemic acute stroke. Studies that investigate paracetamol in addition to other methods that induce hypothermia are scarce [34,35].

It is thus certain that paracetamol is not efficacious in producing or maintaining the targeted temperature in therapeutic hypothermia, but its hypothermic mechanism of action could be of high interest in developing new pharmacological tools for lowering the body temperature.

A comprehensive review on the role of prostaglandins and nonsteroidal anti-inflammatory drugs in the hypothermic response in animals was published by Aronoff D.M. and Romanovsky A.A. in a volume of Sharma H., 2007 [36].

Since then, not many reviews regarding the hypothermic effect of acetaminophen have been performed, leaving much of the new data in the field unsystematised.

The aim of the study is to review the mechanisms of paracetamol-induced hypothermia in rodents or the results issued from in vitro studies using the same species' tissues (in doses that do not produce hepatic impairment) using the latest developments published in scientific journals over the last 15 years (1 January 2007–31 December 2021). The available human studies on this subject will also be analysed.

## 2. Materials and Methods

A literature review of paracetamol's pharmacological influence on body temperature in rodents (mice and rats) was performed using the "tags" shown below to search the PubMed database.

The tag used initially was "(paracetamol OR acetaminophen) AND (hypothermia OR hypothermic OR antipyretic OR pyrexia OR "body temperature")", and it resulted in 20,688 articles (see Figure 1). When using the filter "mice OR rats", the search returned 4669 results.

The final, slightly more restrictive tag used was as follows:

"(paracetamol OR acetaminophen) AND (hypothermia OR hypothermic OR "body temperature") AND (mice OR rats)", limits 1 January 2007–31 December 2021.

The search returned 44 results.

Our purpose was to evaluate in rodents the possible hypothermic mechanism of non-toxic doses of paracetamol (excluding paracetamol-induced hepatic toxicity).

After reading the full text, we excluded 24 articles that did not present the mechanism of action for the hypothermic or antipyretic effects of paracetamol, as well as those that dealt with these mechanisms in hepatic insufficiency settings.

Nine articles dealt with the in vivo mechanism of paracetamol hypothermia and five articles were chosen since they presented in vitro experiments using paracetamol (all five articles were already included in the first nine). The results are presented in Tables 1 and 2 and in the Discussion section. An additional 11 articles were also screened and included as remarks in the Discussion. Searching inside the references of articles, we identified two studies in humans regarding paracetamol's hypothermic effect.

**Table 1.** Synthesis of the findings of the literature that presents paracetamol's hypothermic effects in vivo.

| Study, Reference | Subjects' Species; Strain or Transgenic Animals Used; Gender; Groups N | Study Design—Temperature Measurements, Paracetamol Doses, Routes, Substances for Interactions | Results; Authors' Conclusions; Our Remarks |
|---|---|---|---|
| Li S. et al., 2008 [37] | M; C57BL/6J (WT) COX-1 K.O.; G-ns; N = 6 (in all exp) | CBT recorded every 2 min with a thermo-couple inserted 2 cm into the colon and taped to the tail, plus a thermometer | |
| | | P 80, 120, 140, 160 mg/kg, i.v.<br>LPS—E. coli used for inducing fever, P administered 1 h before and 1 h after LPS. | Hypothermia registered at P 160 mg/kg in both WT and COX-1 KO M.<br>LPS 1 h after P 80, 120, 140 mg/kg ↑ CBT as in veh; LPS following P160 mg/kg adm ↑ CBT in hypothermic M.<br>P 80 and 160 mg/kg, 1 h after LPS-induced fever, ↓ CBT.<br>↓ CBT in COX-1 K.O. M comparable with ↓ showed in WT M in P 80 mg/kg 1 h after LPS. |
| | | | P hypothermia induced in non-febrile mice did not involve COX-1 |
| Corley G. et al., 2009 [38] | R; Sprague–Dawley G-m; N = 6–11 (in all exp) | CBT measured with a thermistor probe, inserted 6 cm into the rectum, plus a digital thermometer. | |
| | | (a) P 100, 250, 375, 500 mg/kg i.p.<br>(b) Naltrexone (μ receptor antagonist) 10 mg/kg sc, 2 adm.<br>Naltrindole (δ receptor antagonist), 1 mg/kg s.c.<br>Nor-BNI (κ receptor antagonist) 10 mg/kg i.p.<br>SR 141716A (CB1 receptor antagonist), 5mg/kg i.m.<br>JTC-801 (NOP receptor antagonist) 1mg/kg, i.p.,<br>P400 mg/kg (after antagonists adm, except of naltrexone adm in 2 doses prior and after P). | (a) Only the highest dose of P (500 mg/kg) ↓CBT.<br>(b) P 400 mg/kg alone produced hypothermia that was not significantly different from the hypothermia produced by each of μ/k/δ antagonists, MOP antagonist, or CB1 antagonist + P 400 mg/kg. |
| | | | Opioid, MOP, and CB1 receptors were not involved in P-induced hypothermia. |
| Briyal S. et al., 2010 [39] | R; Sprague–Dawley G-m; N = 6 (in each group) | CBT measured using a thermometer with colonic probe. | |
| | | P 300 mg/kg, i.p. + BQ123 (endothelin receptor antagonist) 1 mg/kg, i.v.<br>A rat model of MCA occlusion was used. Substances were administered three times:<br>(1) at 30 min after MCA occlusion,<br>(2) at the time of reperfusion, and<br>(3) 2 h after reperfusion. | P 300 mg/kg, i.p. alone produced a ↓ CBT, and PARA + BQ123 produced an even greater hypothermic response in R (41%) when compared with the adm of P alone. |
| | | | BQ123 enhanced the hypothermic effect of P and may reduce the lesions following cerebral ischemia |

**Table 1.** *Cont.*

| Study, Reference | Subjects' Species; Strain or Transgenic Animals Used; Gender; Groups N | Study Design—Temperature Measurements, Paracetamol Doses, Routes, Substances for Interactions | Results; Authors' Conclusions; Our Remarks |
|---|---|---|---|
| Ayoub S.S. et al., 2011 [40] | M; C57BL/6 and COX-1 K.O: and COX-2 K.O: Biozzi ABH, ABH CB1 receptor K.O. and FAAH K.O. and TRPV1 K.O. G-m; N = 5–6 for each experiment | BT was measured with a thermocouple probe placed under the hindlimb. | |
| | | (a) CB1 receptor K.O. M treated with P 300 mg/kg i.p. | (a) CB1R K.O. M treated with P 300 mg/kg i.p. ↓ BT after 1 h. The effect is consistent with those seen in C57BL/6 M. |
| | | (b) C57BL/6 M treated with AM251 (CB1 receptor antagonist) i.p. 5 mg/kg, or/and P 300 mg/kg; AM251 5 mg/kg and WIN55212,2 (CB1/CB2 receptor agonist) 20 mg/kg i.p. | (b) AM251 inhibited the hypothermic response induced by WIN55212,2. AM251 did not prevent the ↓ of BT produced by P. |
| | | (c) TRPV1 K.O. M treated with P 300 mg/kg i.p. | (c) TRPV1 K.O. M treated with P 300 mg/kg i.p. ↓ BT after 1 h. The hypothermic effect of P is not mediated through TRPV1 receptors. |
| | | (d) C57BL/6 M treated with SB366791 (TRPV1 antagonist) 2 mg/kg i.p. or veh + (I) Capsaicin (TRPV1 agonist) 1 mg/kg s.c. or (II) P 300 mg/kg i.p. | (d) SB366791 + P 300 mg/kg i.p. ↓ BT as in animals treated with P alone. SB366791 produced a 2 °C reversal of capsaicin hypothermia. |
| | | (e) C57BL/6 M treated with AM404 (a metabolite of P that also activates the endocannabinoid system) at doses of 10, 20, and 40 mg/kg i.p. | (e) AM404 adm alone did not induce hypothermia. |
| | | (f) C57BL/6 M treated with: URB597 (a selective inhibitor of FAAH) 0.3 mg/kg i.p. (pretreated) or veh. + (I) Anandamide (principal endocannabinoid) 5 mg/kg i.p. or (II) P 300 mg/kg i.p. | (f) AM404 pathways do not mediate the hypothermic effect of P. |
| | | (g) FAAH (fatty acid amide hydrolase) K.O. M treated with P 300 mg/kg i.p. compared with C57BL/6 treated with P 300 mg/kg i.p. | (g) P ↓ BT (measured 1 h after adm) to the same extent in FAAH K.O. M, as well as in C57BL/6 M. FAAH pathways do not mediate the hypothermic effect of P. |
| | | (h) C57BL/6 M treated with P 200 mg/kg i.p. or WIN55-212,2 5 mg/kg i.p. or WIN55-212,2 5mg/kg i.p. + P 200 mg/kg i.p. | (h) WIN55-212,2 + P 200 mg/kg resulted in ↓ BT by 5.75 and 9.25 °C after 0.5 and 1 h, respectively, compared with veh. P in lower doses co-administered with WIN55-212,2 induces additive hypothermia. |
| | | | Five findings result from this article:<br>– The pharmacological inhibition of FAAH did not reduce the hypothermic effect of P;<br>– FAAH K.O. M that received P presented a similar hypothermic effect to that recorded in WT M;<br>– The CB1 antagonist and TRPV1 antagonist did not reduce the hypothermic effect of P;<br>– When using K.O. M, CB1R K.O. M, or TRPV1 K.O. M, the hypothermic effect was similar to that recorded in WT M; and<br>– P and WIN55-212,2 induce additive hypothermia. |

**Table 1.** *Cont.*

| Study, Reference | Subjects' Species; Strain or Transgenic Animals Used; Gender; Groups N | Study Design—Temperature Measurements, Paracetamol Doses, Routes, Substances for Interactions | Results; Authors' Conclusions; Our Remarks |
|---|---|---|---|
| Gentry S. et al.,2015 [41] | M; C57BL/6 and TRPA1 K.O. TRPV1 K.O. and TRPA1 and TRPV1 K.O. (double knockout) G-f and m N = 6–12 for each exp | CBT was measured with a chip incorporated with a thermometer subcutaneously implanted in the shoulder of M. | |
| | | (a) C57BL/6 M and TRPA1 K.O. M treated with P 300 mg/kg s.c. | (a) P 300 mg/kg s.c. ↓ CBT in C57BL/6 M, but not in TRPA1 K.O. M. |
| | | (b) C57BL/6 M treated with P 100 μg intrathecally. | (b) P 100 μg intrathecally did not reduce CBT but produced analgesia. Hypothermia does not contri-bute to the spinal analgesia in M. |
| | | (c) C57BL/6 M and TRPA1 K.O. M prior treated with RTX (TRPV1 agonist), 6.3 μg/kg s.c. recieved P 300 mg/kg s.c. | (c) RTX 6.3 μg/kg s.c. provided a prolonged ↓ CBT both in C57BL/6 and TRPA1 K.O. Both TRPV1 and TRPA1 receptors are involved in the hypothermic response of P. |
| | | (d) C57BL/6 M treated with indomethacin (nonselective COX1 COX2 inh) 10 mg/kg s.c. + P 300 mg/kg s.c. | (d) Indomethacin did not modify CBT. Indomethacin + P did not inhibit the hypothermic effect of P. P hypothermic effect acts independently of COX-1 and COX-2. |
| | | (e) C57BL/6 M treated with CHEM 5861528 (TRPA1 antagonist) 40 mg/kg p.o., CHEM 5861528 100 mg/kg p.o., or CHEM 5861528 300 mg/kg p.o. Then, P 300 mg/kgc s.c. | (e) CHEM5861528 inhibited the hypothermic effect of P in a dose-dependent manner. The highest dose, 300 mg/kg p.o., abolished the hypothermic effect of P. |
| | | (f) C57BL/6 M treated with BCTP (TRPV1 antagonist) 30 mg/kg p.o., then P 300 mg/kg s.c. C57BL/6 and TRPV1 K.O. treated with P 300 mg/kg s.c. | (f) The CBT was not different in C57BL/6 M and TRPV1 K.O. M before the exp. TRPV1 antagonist BCTP 30 mg/kg p.o. administered in C57BL/6 M ↑ CBT, but did not inhibit the hypothermia induced by the adm of P 300 mg/kg s.c. P 300 mg/kg s.c. identically ↓ CBT both in C57BL/6 M and TRPV1 K.O. M. |
| | | (g) C57BL/6, TRPV1 K.O, TRPA1 K.O., TRPA1 and TRPV1 K.O. (double-knockout) M were treated with yeast s.c. to induce pyrexia (1 °C) and then with P300 mg/kg s.c. | (g) Yeast induced similar ↑ CBT in C57BL/6, TRPV1 K.O., TRPA1 K.O., and in TRPA1 and TRPV1 K.O. (double-knockout) M. P 300 mg/kg s.c. induced hypothermia in C57BL/6 M and TRPV1 K.O. M, but acted only as an antipyretic in TRPA1 K.O. M and to the TRPA1 and TRPV1 K.O. (double K.O.) M, restoring CBT to the pre-yeast adm temperature. |
| | | (h) C57BL/6 M and TRPA1 and TRPV1 double K.O. M treated with yeast s.c. + indomethacin 30 mg/kg s.c. + P 300 mg/kg s.c. | (h) Indomethacin restored CBT to the time of pre-yeast adm both in C57BL/6 M and TRPA1 and TRPV1 double K.O. M Indomethacin 30 mg/kg s.c. + P 300 mg/kg resulted in hypothermia in C57BL/6 M, but not in TRPA1 K.O. M and TRPV1 K.O. M. In TRPA1 K.O. M and TRPV1 K.O. M, the temperature decrease continued later with a small reduction in CBT both in indomethacin + P, as well as indomethacin + veh. |
| | | | TRPV1 is not involved in P-induced hypothermia. TRPA1 mediates P-evoked hypothermia. |
| Ahangar N. et al., 2015 [42] | R; Sprague–Dawley G-m; N = 7 for each exp. | CBT—measured with a digital thermometer lubricated and inserted 4 cm into the rectum. | |
| | | (a) P 100, 200, or 300 mg/kg i.p. | (a) P produces a dose- and time-dependent hypothermic effect. A suboptimal dose of P was then chosen for the next exp. |
| | | (b) P 200 mg/kg i.p. or P 200 mg. kg i.p. + flumazenil (BZD receptor antagonist, part of GABAA receptor) 10 mg/kg i.p. or P 200 mg/kg i.p. + picrotoxin ($GABA_A$ antagonist) 2 mg/kg i.p. | (b) The combination of P 200 mg/kg i.p. with flumazenil 10 mg/kg i.p. reduced the hypothermic effect of P. The addition of picrotoxin 2 mg/kg i.p. in R treated with P 200 mg/kg i.p. reduced the hypothermic effect of P. |
| | | | P's hypothermic effect might be mediated (in part) through the $GABA_A$ receptors. |
| Fukushima A. et al., 2017 [43] | M; Ddy 4–6 weeks old. G-n.s. N = 10–20 for each exp involving P | CBT was measured with a digital thermometer linked with a probe inserted 25 mm into the rectum. | |
| | | (a) PCPA 300 mg/kg i.p. for 5 days + P 200 mg/kg i.p. or P 300 mg/kg i.p. (b) Cyproheptadine hydrochloride (H1 blocker with serotonin properties) 0.3 mg/kg, i.p. + P 200 mg/kg i.p. fluoxetine hydrochloride (serotonin-specific reuptake inhibitor) 3 mg/kg, i.p. + P 200 mg/kg i.p. | (a) P 300 mg/kg i.p. significantly ↓ CBT in PCPA group. P200 mg/kg i.p. had no significant effect on CBT. (b) The combination of fluoxetine with P or of cyproheptadine with P did not influence the hypothermic effect of P. |
| | | | The hypothermic effect of P is not mediated by the serotoninergic system. |

**Table 1.** *Cont.*

| Study, Reference | Subjects' Species; Strain or Transgenic Animals Used; Gender; Groups N | Study Design—Temperature Measurements, Paracetamol Doses, Routes, Substances for Interactions | Results; Authors' Conclusions; Our Remarks |
|---|---|---|---|
| Mirrasekhian et al., 2018 [44] | M; C57BL/6 and TRPA1 K.O. G-f and m N = 4–20 for each exp | CBT was measured with a digital thermometer linked with a probe inserted 25 mm into the rectum. | |
| | | (a) C57BL/6 M treated with P 100, 150, or 200 mg/kg i.p. | (a) P 200 mg/kg i.p. produced hypothermia, whereas P100 and 150 mg/kg i.p. did not. |
| | | (b) TRPA1 K.O. M treated with P 200 mg/kg i.p. | (b) P200 mg/kg i.p. adm in C57BL/6 M produced hypothermia, but not in TRPA1 K.O. M. |
| | | (c) C57BL/6 treated with NAC (precursor of glutathione, NAPQI scavenger) 1 g/kg + P 200 mg/kg | (c) NAC decreases the hypothermic effect of P. |
| | | (d) C57BL/6 M and TRPA1 K.O. M treated with LPS 100 µg/kg i.p. (pretreatment) + P 150 mg/kg i.p. | (d) P150 mg/kg does not elicit hypothermia, but does normalize body temperature after LPS administration in both C57BL/6 M and TRPA1 K.O. M.<br>The P group suffered an important, but transient, ↓ CBT. |
| | | | TRPA1 receptors might not be involved in the antipyretic effect of P.<br>P hypothermia could involve NAPQI. |
| Ayoub S.S. et al., 2019 [45] | M; C57BL/6J and COX-1 K.O.; G-m; N = 3–5 for each exp | BT—measured with temperature-sensitive transponders, implanted s.c., and with a sensitive scanner held 3 cm above the back of the M. To obtain a significant pyrexia, M were transferred into a warm air system at 30 °C, and after 1h, LPS was adm i.p. | |
| | | (a) C57BL/6J treated with LPS 10 µg/kg i.p and then received: SC560 15 mg/kg i.p. (COX 1 inhibitor), Celecoxib 15 mg/kg i.p. (COX 2 inhibitor), or P 300 mg/kg i.p. | (a) P 300 mg/kg i.p. induced significant hypothermia, with ↓ CBT with a decrease of 2.5 °C after 0.5 h and 3.63 °C after 1 h.<br>Neither SC560 (15 mg/kg) or celecoxib (15 mg/kg) had hypothermic effects. |
| | | (b) WT and COX-1 K.O. M were treated with P 200 mg/kg s.c., then treated with LPS 10 µg/kg i.p. compared with<br>WT and COX-1 K.O. M were treated with indomethacin (non-selective dual COX-1/COX-2 inhibitor) 5 mg/kg s.c. and 10 mg/kg s.c. LPS 10 µg/kg i.p. | (b) Prophylactic adm of P 200 mg/kg s.c. did not have a hypothermic or antipyretic effect in COX-1 K.O. M when compared to the COX-1 K.O. M treated with veh.<br>The fever observed in COX-1 K.O. M lasted longer than in WT M treated with LPS.<br>Indomethacin did not induce hypothermia (ambient temp = 24 °C), but after pyrexia was induced with LPS, indomethacin 5 mg/kg s.c. ↓ fever induced by LPS (ambient temp = 30 °C). |
| | | (c) WT M and COX-1 K.O. M received LPS 10 µg/kg i.p. to induce pyrexia and then treated with P 200 mg/kg s.c. | (c) In COX-1 K.O. M, P ↓ febrile BT post-LPS non-significantly by approx. 0.67 °C after 1 h, with BT between 35.8 to 35.93 °C.<br>In WT M, P ↓ febrile BT significantly with approx. 4.56 °C after 1 h post-administration.<br>PGE2 synthesis was abolished in COX-1 K.O. M. treated with P. |
| | | | A target for the P-induced hypothermia is not COX-1 and it is likely to be a variant of COX-1. |

Abbreviations: adm—administration, ~ and approx.—approximative, BT—body temperature, BQ123—2-[(3*R*,6*R*,9*S*,12*R*,15*S*)-6-(1*H*-indol-3-ylmethyl)-9-(2-methylpropyl)-2,5,8,11,14-pentaoxo-12-propan-2-yl-1,4,7,10,13-pentazabicyclo[13.3.0]octadecan-3-yl]acetic acid, BTCP—7-tert-butyl-6-(4-chloro-phenyl)-2-thioxo-2,3-dihydro-1H-pyrido[2,3-d]pyrimidin-4-one, CBT—core body temperature, CHEM5861528—2-(1,3-Dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-7H-purin-7-yl)-N-[4-(1-methylpropyl)phenyl] acetamide, CMC—carboxymethylcellulose sodium solution, exp—experiment(al), COX—cyclooxygenase, DMSO—dimethyl sulfoxide, G—gender: m—male, ns—not specified, h—hour, i.m.—intramuscular, inh—inhibitor, i.p.—intraperitoneal, i.v.—intravenous, K.O.—knock-out, LPS—lipopolysaccharides, M—mice, MCA—middle cerebral artery, min—minute, N—number of animals per group, NAC—N-Acetyl cysteine, NAPQI—N-acetyl-p-benzoquinone imine (a reactive metabolite of paracetamol), Nor-BNI—Nor-binaltorphimine, P—paracetamol, p.o.—per os, PCPA—DL-p-chlorophenylalanine, PGE2—prostaglandin E2, PGF2α—prostaglandin F2α, 6-keto-PGF1α—6 keto-prostaglandin 1 α, R—rats, RTX—resiniferatoxin, s.c.—subcutaneous, SC560—5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-(trifluoromethyl)-1H-pyrazole, temp.—temperature, TRPA1—transient receptor potential cation channel subfamily A member 1, TRPV1—transient receptor potential cation channel subfamily V member 1, TXB2—thromboxane B2, veh—vehicle, WT—wild type, ↑—increased, ↓—decreased.

**Table 2.** Synthesis of the findings of the literature that present paracetamol's hypothermic effects in vitro/ex vivo.

| Study; Reference | Experimental Model | Species, System, Analysis | Treatment | Effects |
|---|---|---|---|---|
| Li S., 2007 [37] | "ex vivo" PGE2 concentration evaluation | M blood, brain; enzyme immunoassay kit was used | P 160 mg/kg i.v. | P did not influence brain and plasma levels of PGE2 of non-febrile WT M at 1, 2, or 3 h after adm<br>P produced an immediate ↓ of the elevated brain PGE2 level of M treated with LPS to its basal value within 1 h (2 h after LPS); PGE2 then ↑ to its pre-P high level in 1 h. |
| Briyal A., 2010 [39] | "ex vivo" evaluation of MDA and GSH activity in the brain | R brain | P 300 mg/kg, i.p. + BQ123 (endothelin receptor antagonist) 1 mg/kg, i.v. | P did not but BQ123 alone and in combination with P ↓ MDA and ↑ GSH levels in ischaemic rats with MCA occlusion, and combination of P and BQ123 was more effective in reducing the neuronal damage following cerebral ischaemia. |
| Ayuob S.S., 2011 [40] | "ex vivo" evaluation of PGE2 concentrations in the brain | C57BL/6, CB1R K.O. M brain, an enzyme immunoassay kit was used | P 300 mg/kg i.p. | The brain PGE2 concentration of CB1R K.O. M was also compared 1 h after 300 mg/kg P or veh treatments. Paracetamol reduced brain PGE2 levels in CB1R K.O. M compared to veh-treated M. |
| Mirrasekhian E., 2018 [44] | "ex vivo" evaluation of PGE2, PGF2α, 6-keto-PGF1α (a stable metabolite of PGI2), and TXB2 (a stable metabolite of TXA2) | M blood, brain; HPLC system coupled to a tandem mass spectrometer | LPS (100 µg/kg i.p.) or saline was administered 4 h prior to i.p. injection of P (150 mg/kg) or veh | P completely suppressed the LPS induced ↑ of PGE2 in the brain, and ↓ the levels of some other prostanoids in brain and in blood. |
| Ayoub S.S., 2019 [45] | (a) "ex vivo" evaluation of PGE2 concentrations in the brain<br>(b) evaluation of P plasma concentration | (a) M blood, brain; enzyme immunoassay kit was used;<br>(b) the adm of P to WT M (C57BL/6 J); the concentration of P was measured using a colorimetric method | (a) 200 mg/kg P i.p.<br>(b) s.c. adm of 200 mg/kg P, plasma was collected from M at 0.5, 1, 2, 3, and 4 h | The comparisons of the effect of 200 mg/kg P on hypothalamic PGE2 levels 1 h after P adm (and 3 h after LPS), between COX-1 WT M and COX-1 K.O. M showed a decrease in PGE2 in W.T. M but not in transgenic M, signifying that LPS-induced fever is PGE2-mediated and COX-2-dependent<br>At the dose of 15 mg/kg, SC560 (COX-1 blocker without hypothermic action, independent of doses used) reduced the brain PGE2 synthesis by 76%. |

Abbreviations: adm—administration, BQ123—2-[(3*R*,6*R*,9*S*,12*R*,15*S*)-6-(1*H*-indol-3-ylmethyl)-9-(2-methylpropyl)-2,5,8,11,14-pentaoxo-12-propan-2-yl-1,4,7,10,13-pentazabicyclo[13.3.0]octadecan-3-yl]acetic acid, CB1R—cannabinoid CB1 receptors, COX—cyclooxygenase, GSH—glutathione, h—hour, HPLC—high performance liquid chromatography, i.m.—intramuscular, inh—inhibitor, i.p.—intraperitoneal, i.v.—intravenous, K.O.—knock-out, LPS—lipopolysaccharides, M—mice, MCA—middle cerebral artery, MDA—malonyldialdehyde, min—minute, P—paracetamol, PGE2—prostaglandin E2, PGF2—α-prostaglandin F2α, 6-keto-PGF1α—6 keto-prostaglandin 1 α, R—rats, s.c.—subcutaneous, SC560—5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-(trifluoromethyl)-1H-pyrazole, TXB2—thromboxane B2, veh—vehicle, WT—wild type, ↑—increased, ↓—decreased.

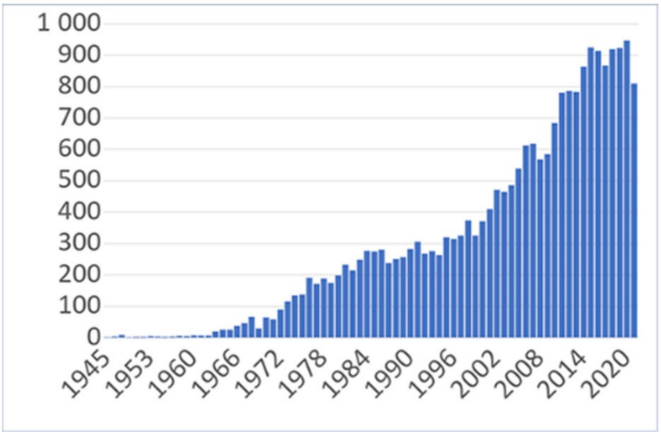


**Figure 1.** The evolution of interest regarding paracetamol's hypothermic and antipyretic effects as reflected in the number of articles published and indexed in PubMed between 1945 and 2021.

## 3. Results

The interest regarding paracetamol's hypothermic and antipyretic effects is in a positive trend reflected in the number of articles on this subject published and indexed in PubMed between 1945 and 2021 (See Figure 1).

An overview of the data found in the previously mentioned scientific experimental works was depicted in Tables 1 and 2.

## 4. Discussion

### *4.1. Considerations Regarding the Methods Used in the Studies Analysed*

The mechanism of hypothermia observed after paracetamol's hepatic impairment was not analysed because hypothermia appearing in this condition could be induced by hepatic pathology, not by a direct action of paracetamol, and it could induce some biases in the research. From another point of view, we aimed to find potential therapeutic strategies in which inducing hypothermia is necessary in different conditions, such as CNS severe hypoxia. In some cases, hypothermia associated with hepatic impairment is a pathology by itself and cannot be used as a therapeutic method.

Mice and rats have different body temperature regulations and different dose thresholds for paracetamol toxicity. Rats are more resistant to paracetamol-induced liver injury, as the LD50 (lethal dose) for rats is 2404 mg/kg body weight (bw), whereas for mice it is 338 mg/kg bw for the oral route of administration [46].

The temperature measurement methods were different across studies. Some used CBT measurements (intrarectally or by subcutaneous implants, although the last is not a proper CBT measurement), and others used peripherally measured temperature under the hind limb. Moreover, the ambient temperature was also different across studies, and sometimes the temperature was used in disregard of the purpose of the study. For example, some authors used the ambient temperature of 23 °C instead of 30 °C, as in the study in which lipopolysaccharides (LPS) were used to induce pyrexia, although an ambient temperature of 30 °C represents the working standard [37].

These variabilities made it impossible for us to superpose the results of different labs and researchers.

### *4.2. Lack of Hypothermic Effect after COX-1 or/and COX-2 Inhibition, but Possibly after Inhibition of (Some) Cyclooxygenase-1 Variant Enzymes*

A number of studies showed that paracetamol-induced hypothermia is correlated with reduced brain PGE2 synthesis [9]. The hypothermic effect in mice is established after 30 min at therapeutic doses of paracetamol (160 mg/kg bw) [37].

The hypothermic action of paracetamol appeared to be linked to the inhibition of a COX-1 variant (probably constitutive), whereas the antipyretic action may be induced by

the COX-2 inhibition [47]. Li S. et al., 2008, reported the antipyretic action of paracetamol one hour after LPS administration in COX-1 knockout mice (although, theoretically, it lasts at least 2 h until COX-2 induction by LPS occurs through processes involving the nucleus of cells, COX-2 being an inducible enzyme) [37].

Paracetamol did not change the PGE2 levels in plasma or in the brain of the non-febrile mice, leading to the conclusion that its hypothermic effect is probably not due to its COX-2-inhibiting activity. This effect might be due to the anti-glutamate or antioxidant activities of paracetamol [37]. These results conflict with the hypothesis of Ayoub S.S. et al., 2006, that mention an involvement of COX-1, COX-2 and a hypothetic COX-3 isoenzymes [9].

The hypothermic effect of paracetamol is due to the inhibition of (some) cyclooxygenase-1 variant enzymes, after Ayoub S.S. and Flower R.J., 2019 [45]. Hypothermia induced by paracetamol depends indeed on a COX gene in mice, but its translation product might be different from COX-1 and COX-2 [45]. These results are based on the lack of a hypothermic effect of a COX-1 inhibitor (SC-560) or a COX-2 inhibitor (celecoxib), respectively. The non-selective COX-1 and COX-2 inhibitor indomethacin produced no hypothermia, only a robust antipyretic effect in mice when LPS was administered [45].

The reduction of paracetamol-induced hypothermia in COX-1 K.O. mice was accompanied by a reduction in the paracetamol-induced inhibition of brain PGE2 synthesis [48,49].

LPS treatment induced PGE2 in the brain, and this induction was completely suppressed by paracetamol. Paracetamol also reduced the levels of several other prostanoids in the brain, as well as in the blood. This fact signifies that paracetamol not only blocked induced PGE2, implying an effect on COX-2, but also reduced the levels of other prostaglandins and TXB2. This fact indicates (after Mirrasekhian E. et al., 2018) that paracetamol inhibits both COX-1 and COX-2 and may reveal that paracetamol interacts with the peroxidase site of the cyclooxygenase enzymes, present in both isoforms. This interaction is preferentially seen in tissues with low peroxide concentration, such as the brain [44].

### *4.3. Trials in Humans Concerning Hypothermia after Paracetamol*

Acute paracetamol ingestion (at a dose of 20 mg/kg lean body mass) reduces normal, non-febrile CBT during sub-neutral conditions for thermoregulation, e.g., 120 min passive exposure to 20 °C, 40% relative humidity [50]. The authors conclude that this reduction of CBT induced by paracetamol is due to a decrease of autonomic shivering responses, probably mediated by COX-2 inhibition. In 2008, Hinz et al. found that a standard dose of paracetamol caused an almost complete inhibition of COX-2 in humans, whereas a moderate inhibition of COX-1 was noticed [51]. In accordance with the former data, the same research group [52] found that the same doses of paracetamol reduced CBT (0.16–0.57 °C) after 120 min exposure during acute cold stress (10 °C) but had no effect on CBT at a neutral ambient temperature (25 °C).

Taking into account these results in humans, we can explain the hypothermic effect of paracetamol in mice as being due to the ambient temperature under 30 °C used in the experiments. A temperature of 30 °C is the lowest level that does not produce thermogenesis in mice [53].

### *4.4. The Serotoninergic, Opioid, Nociceptin, Cannabinoid, Endothelin, GABA-ergic Systems, and Mitochondrial-Related Functions Involvment in Paracetamol's Hypothermic Effect*

Fukushima A. et al., 2017, showed no effect on influencing the hypothermic effect of paracetamol when pre-treating mice with the serotonin synthesis inhibitor DL-p-chlorophenylalanine (PCPA), thus producing a decrease of serotonin in the brain by 70% and significantly inhibiting the analgesic action of paracetamol. They concluded that the hypothermic effect of paracetamol is not mediated by the serotonin system, in contrast with its analgesic effect [43].

The 5-$HT_7$ receptor is a potential defense mechanism in stopping fever, but the antipyretic property of paracetamol is not due to its action on 5-$HT_7$ receptors [54].

The opioid system seems not to be involved either, as Corley G. et al., 2009, showed in rats the lack of effects of opioid receptor antagonists (μ, k, or δ) on paracetamol-induced hypothermia. The authors also demonstrated no effect of cannabinoid CB1 or nociceptin (NOP) receptor antagonists [38].

There is, however, a potentiation of paracetamol's hypothermic effect when it is co-administered with an endothelin receptor antagonist (the substance codenamed BQ123), as demonstrated by Briyal S. et al., 2010 [39]. This effect might reduce the infarction volume in rats following cerebral ischemia.

The levels of malonyldialdehyde (MDA) increased and glutathione (GSH) decreased in the brains of rats following ischemia. Paracetamol alone did not influence these enzymes. BQ123 alone and in combination with paracetamol decreased MDA and increased GSH levels in the brains of ischaemic rats. MDA levels significantly improved following treatment with both BQ123 and paracetamol compared to BQ123 alone. A decreased level of MDA in the brain of ischemic rats indicates a decrease in the levels of lipid peroxidation. An increase in GSH levels (signifying neuroprotection) in the brain of ischaemic rats treated with BQ123 and paracetamol occurred. This suggests that hypothermia induced by BQ123 plus paracetamol can be responsible for protecting the brain against the oxidative stress in the middle cerebral artery occlusion model [39].

Aydin M. et al., 2011, describes in immature rats that paracetamol decreased some markers of cellular injuries (apoptosis, heat shock protein 70 – HSP70) and increased healthy cell counts after hyperthermia induced by LPS in the brain [55].

A novel explanation of paracetamol-induced hypothermia in rodents is provided by Bashir S. et al., 2020. Paracetamol significantly attenuated mitochondrial function by up to 30% for complex I and 40% for complex II, in vitro. These data suggest that both the antipyretic and hypothermic effects induced by paracetamol could be attributed to the direct inhibition of lipolysis and mitochondrial function [56].

The cannabinoid hypothesis of the hypothermic effect of paracetamol (via the metabolite AM404 synthesised under the influence of fatty acid amide hydrolase, FAAH) is unsupported by at least four findings [40]:

- The pharmacological inhibition of FAAH did not reduce the hypothermic effect of paracetamol;
- FAAH K.O. mice that received paracetamol presented a similar hypothermic effect to that recorded in wild type (WT) mice;
- The CB1 and TRPV1 antagonists did not reduce the hypothermic effect of paracetamol; and
- When using K.O. mice, CB1R K.O., or TRPV1 K.O., the hypothermic effect was similar to that recorded in WT mice.

However, Ayoub S.S. et al., 2011, made an interesting finding regarding the cannabinoid system and the hypothermic effect of paracetamol: when used in combination with WIN55,212-2—an agonist of CB1 and CB2 receptors—paracetamol demonstrated a supra-additive hypothermic effect [40].

The hypothermic effect of paracetamol was antagonized by picrotoxin (a $GABA_A$ receptor antagonist) and flumazenil (a benzodiazepine receptor antagonist). Paracetamol's hypothermic effect might be mediated somehow through $GABA_A$ receptors [42].

Paracetamol reduced brain PGE2 levels in CB1 receptor K.O. mice in a way comparable to non-treated mice of the same strain. This fact is further proof of the lack of CB1 receptors involvement in the induction of hypothermia by paracetamol [40].

### *4.5. Anti-Hyperthermic or Antipyretic Effects of Paracetamol*

Paracetamol, in addition to its demonstrated hypothermic effect at therapeutic (160–200 mg/kg bw) or slightly higher doses (300 mg/kg bw) has been used in vivo at doses of 300 mg/kg bw to interfere with TRPV1 antagonists (the substance codenamed AMG8163, which causes hyperthermia in mice). Paracetamol 300 mg/kg bw decreased body temperature by 1.5 °C, completely reversing hyperthermia. An interesting fact is that

low doses of paracetamol (100 or 150 mg/kg) were not effective in reversing AMG8163-induced hyperthermia [57]. It is possible that the same mechanism of action that produces hypothermia can be involved in diminishing hyperthermia induced by TRPV1 antagonists.

In acute stages of fractures in Wistar rats, the administration of paracetamol did not control hyperthermia [58].

Paracetamol partially prevents the febrile response of the body induced by endogenous pyrogens, such as IL1β, including the administration of these pyrogens in brain tissues. Csetényi B. et al., 2017, proposed that prostaglandin-mediated mechanisms have an important role in the mechanism of action of IL1β in the cingulate cortex since paracetamol pre-treatment partially prevented an increase in the body temperature of Wistar rats [59].

On the contrary, paracetamol did not influence the pyrogenic effect of IL1β injection in the nucleus accumbens of Wistar rats, and it seemed that this nucleus is not involved in paracetamol's antipyretic effects [60].

The ex vivo studies of the research group of Ayoub S.S., 2019, confirmed that LPS-induced fever is PGE2-mediated and COX-2-dependent. At a dose of 15 mg/kg, SC560 (a COX-1 blocker without hypothermic action) reduced brain PGE2 synthesis by 76%. COX-1 inhibition by SC560 did not produce hypothermia by decreasing PGE2 synthesis. The authors also showed that COX-2 inhibition with celecoxib did not produce hypothermia. Since COX-2 K.O. mice fail to develop a fever in response to LPS, COX-2 seemed to be the antipyretic target for paracetamol [45].

The effect of other pyrogens, apart from LPS, could be reversed by paracetamol. Paracetamol (150 mg/kg) reduced the fever associated with zymosan induced experimental arthritis in rats and decreased the concentration of PGE2 in the cerebrospinal fluid, suggesting the involvement of PGE2 in this response [61].

Paracetamol does not interfere with reactive oxygen species (ROS) production, as observed using electron paramagnetic resonance (EPR) studies, although there is an increased formation of ROS in different tissues during LPS-induced fever [62].

### *4.6. TRPA1's Role in Paracetamol-Induced Hypothermia*

As shown above by Ayoub S.S., 2011, TRPV1 K.O. mice have a similar paracetamol-induced hypothermic response as the WT, suggesting that TRPV1 is not involved in the mechanism of hypothermia in paracetamol [40].

These results are also confirmed by Gentry C. et al., 2015, who used TRPV1 K.O., TRPA1 (transient receptor potential cation channel subfamily A member 1) K.O., and WT mice in their studies. Gentry et al. demonstrated that TRPV1 K.O. mice manifested the same hypothermic effect as their WT littermates. Moreover, resiniferatoxin, an agonist of TRPV1 receptors, had no effect on paracetamol's hypothermic effect, thus emphasising the lack of TRPV1 involvement in paracetamol-induced hypothermia [41].

In contrast, in TRPA1 K.O. mice, the administration of paracetamol was without an effect on body temperature. In addition, a TRPA1 antagonist inhibited hypothermia, strongly suggesting that TRPA1 mediates paracetamol-evoked hypothermia.

Moreover, the latest findings indicate that the hypothermic effect might be due to the production of small quantities of NAPQI in the CNS that could stimulate TRPA1 receptors. Nevertheless, it should be noted that NAPQI is synthesised in the presence of an oxidative pathway that could be different in mice, rats, and humans. The large variations in the oxidative enzymes across species should therefore be accounted for when trying to extrapolate the results to humans.

It is thus certain that TRPA1 receptors are involved in the hypothermic effect of paracetamol, but data suggest that they are not responsible for its antipyretic effect. Gentry C. et al., 2015, demonstrated that TRPA1 K.O. mice respond to antipyretic doses of paracetamol [41]. Mirrasekhian E. et al., 2018, also observed that the antipyretic effects obtained at lower doses of paracetamol, insufficient to induce hypothermia, are not dependent on the TRPA1 receptors' stimulation [44]. As noted above, for mice, the hypothermic effect is induced in doses of 160 mg/kg bw, while less than 150 mg/kg bw is required

for inducing an analgesic effect [44,46]. These findings indicate that the hypothermic and antipyretic activities of paracetamol are influenced by TRPA1 and TRPV1 receptors through different mechanisms.

Other drugs from the NSAIDs group were found to induce a hypothermic effect in rodents, e.g., metamizole [63,64].

## 5. Conclusions

We present below the conclusions drawn from the articles analysed in this review. Paracetamol's mechanism of lowering the normal central body temperature is still a subject of debate for researchers. We mention that many of these data are disparate, and some are not confirmed in the further (from a chronological point of view) articles.

- Paracetamol's hypothermic action is due to the inhibition of a COX-1 variant (probably constitutive), and its antipyretic action is due to the inhibition of COX-2;
- Mitochondrial-related functions are involved in paracetamol's hypothermic effect;
- Endothelin receptor antagonists potentiate the hypothermic effect of paracetamol;
- Opioid receptor (μ, k, or δ) antagonists or nociceptin (NOP) receptor antagonists have no effect on paracetamol-induced hypothermia;
- Cannabinoid CB1 receptor antagonists do not influence paracetamol-induced hypothermia;
- Paracetamol has no involvement on the serotoninergic system concerning hypothermia (as opposed to its analgesic effect);
- Paracetamol's hypothermic effect is mediated somehow through $GABA_A$ receptors;
- TRPV1 has no effect on paracetamol-induced hypothermia; and
- TRPA1 is involved in the hypothermic response to paracetamol, possibly via NAPQI, a paracetamol metabolite produced in CNS.

The hypothermic mechanism of paracetamol is different from its antipyretic mechanism. More data is needed, but TRPA1 agonists have the potential to be used in clinical practice to induce hypothermia (for targeted temperature management).

Human studies confirm the in vivo and in vitro experiments in rodents regarding the presence of a hypothermic mechanism after high, non-toxic doses of paracetamol (in sub-neutral ambient temperature and humidity conditions).

Taking into account all these statements, we can observe that paracetamol's hypothermic effect can be regarded in a dual perspective:

- A favorable one, regarding its protective cellular action against brain ischemia; and
- An unfavorable one, regarding its toxicity on mitochondrial function and the inhibition of lipolysis.

Consequently, further research is required to thoroughly understand the mechanisms of action behind paracetamol's hypothermic effect in humans.

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