rabbit genetics

Different drug-processing enzymes with atropine- and cocainesterase activities reported in rabbits and humans

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Abstract. The genetic system involved in atropine and cocaine metabolism in rabbits is different from that implied in humans. In a very simple configuration, two linked autosomal genes located on rabbit chromosome 5 are involved in those two drugs metabolism in this species. A specific atropinesterase activity is missing in humans, but a more complicated cocainesterase activity was reported. Two autosomal genes, *hCE-1* and *hCE-2*, are mainly involved in the metabolism of various esters of cocaine. A butyrylcholinesterase, whose gene is located on human chromosome 3, was also reported in this regard. This review aimed at comprehensively assessing and discussing the atropinesterase and cocainesterase activities in rabbits and humans, with implications in various drugs' metabolism and detoxification.
Key Words: *Oryctolagus cuniculus*, cocaine, atropine, carboxylesterase, cholinesterase.

Introduction. The usual anticholinergic effect of atropine through a parasympatholytic mechanism of action is well-known both in human and veterinary medicine. Although variable effects depend on the species, breed, age, gender and individual factors of reactivity, but also on the dose and route of administration, the general action of atropine is expected: (1) to cause mydriasis, by blocking the parasympathetic innervation of the pupil and ciliary muscle (Mori et al 2019), (2) to increase heart rate, by both central and peripheral stimulation (Kottmeier & Gravenstein 1968), (3) to decrease the salivary secretions, which is important during anaesthesia and surgical manipulations (Olson et al 1993), and (4) to produce vasodilatation and to increase the body temperature (Liu et al 2004).

Historically, cocaine was used as local anesthetic with sodium channel blocking properties, in operations of the ear, nose and throat, but also to alleviate physical and mental pain of terminal disease (Brain & Coward 1989; Killam 1993). The stimulatory effect of cocaine on the central nervous system, by inhibiting the uptake of neurotransmitters, such as epinephrine, norepinephrine, dopamine, but also serotonin, was believed to account for the heart rate increasing effect (Killam 1993). Depending on drug concentration and types of cellular receptors, cocaine may produce reactive oxygen species related to possible inflammatory responses (Hargrave et al 2003). It was demonstrated that cocaine-induced hepatotoxicity, including here hepatic necrosis, is generated by N-oxidative metabolites resulted, for example, from the oxidation of norcocaine and N-hydroxynorcocaine (Roth et al 1992). Myocardial infarctions and embolic strokes by stasis and/or the cardiac endothelial damage were studied in humans, while the enhanced platelet reactivity as a cause for cardiac thrombi also included studies on the rabbit (Killam 1993).

Efforts for decreasing the toxicity of various compounds are permanently made by the organisms. In the cases of the atropine and cocaine, defense mechanisms include reactions of ester-hydrolysis, which form inactive metabolites, easily to be excreted.

In some, but not all rabbits and even in species of plants, atropinesterase was reported to have an influence on the hydrolysis of atropine to tropine and tropic acid (Jindra et al 1963; Liebenberg & Linn 1980; Margolis & Feigelson 1963; Sawin & Glick 1943). This is a non-specific carboxylesterase also involved in the metabolism of other

ester-type drugs, such as procaine (Forster & Hannafin 1979), for example, although the procainesterase activity is known and even involved in atropine metabolism (Greene & Wade 1968).

Atropinesterase activity in humans does not reach the same level as in rabbits. Small amounts of atropine hydrolysis may occur in human body, but they are rather due to the intervention of other carboxylesterases, such as cocainesterase, for example (Harrison et al 2006), which exist in both rabbits and humans. Van Zutphen (1974) even discussed the activity of atropinesterase to be dependent on the presence of cocainesterase in rabbits. Cocainesterase is not necessary for the hydrolyse atropine in rabbits (Mindel 2006), due to the atropine specific metabolism.

Regarding the detoxification from cocaine, this specifically involves cocainesterase activity in a process of hydrolysis into two inactive metabolites, benzoylecgonine and ecgonine methyl ester, both unable to cross the blood-brain barrier (Bosron & Hurley 2002).

In the context of all previously discussed aspects, the aim of this paper was to review certain genetic and physiological aspects of the cocainesterase activity encoding genes, synthesis and action in both rabbits and humans, in relation to the absence of a specific atropinesterase in humans.

Material and Method. Different aspects of genes with cocainesterase and atropinesterase activities in rabbits and humans were mainly discussed here, in a comprehensive presentation. This is a review study based on 35 scientific papers selected on the basis of the criteria of scientific relevance for the subject matter.

Results and Discussion

A brief overview on atropine and cocaine metabolism, and the linked position of encoding genes in the rabbit. Atropine and cocaine are ester-type drugs metabolised by enzymes of the carboxylesterases class through a general process of hydrolysis. These enzymes determine the hydrolysis of various types of esters and amide bonds of both endogenous and exogenous chemicals, forming more soluble compounds such as carboxylic acid and an alcohol or a thiol or an amine (Cashman et al 1996).

The hydrolysis of atropine to tropine and tropic acid is well-known (Kirchhoff et al 2004), although its metabolism still has to be understood. The primary urinary metabolite, the benzoylecgonine, is known to be formed as a result of the cocaine hydrolysis (Bencharit et al 2003), although ecgonine was also considered as urinary metabolite (Stewart et al 1977). A way of cocaine transesterification with ethanol, forming the toxic metabolite cocaethylene, was also discussed by Bencharit et al (2003).

In rabbits, two specific enzymes act in atropine and cocaine metabolism. The gene for atropinesterase encoding, initially designated as *"As"* (Sawin & Glick 1943), was later discovered to be a part of a linkage group, together with the gene for cocainesterase. The two genes were noted with *Est-1* and *Est-2* and are involved in the encoding of cocainesterase and atropinesterase enzymes, respectively (Forster & Hannafin 1979; Fox & Van Zutphen 1977, 1979). The genes of these enzymes were designated as a part of rabbit linkage group (LG) VI (Sawin & Glick 1943), assigned on the q arm of chromosome 5. A homology with the q arm of human chromosome 16 was also reviewed (Korstanje et al 2001). More information about linkage relationships of *Est-2* gene were reviewed by Grădinaru (2021).

In humans, no specific atropinesterase activity was reported in my findings. The metabolism of various esters of cocaine and other drugs involves both *hCE-1* and *hCE-2* enzymes, as members of the carboxylesterase family (this subject is a part of the next discussion).

Specific roles in humans of carboxylesterase family, and not only, for some drugs metabolism. As reviewed by Satoh (2005), carboxylesterases (CarbEs, aliesterases, B esterases, EC 3.1.1.1) together with non-carboxylesterases -

acetylcholinesterase (*AChE*, EC 3.1.1.7) and mammalian paroxonase (arylesterase *PON1*, A esterase, EC 3.1.8.1) - are a part of the α,β-hydrolase-fold family.

Carboxylesterases are widely distributed in different tissues of mammalian species; they are located in the endoplasmic reticulum, with an important activity at the level of the liver but activities were also observed in other tissues such as gut, testis, kidney, skin and blood (Cashman et al 1996; Satoh 2005; Williams 2008). They are serine hydrolases since their active site of serine, in a sequence of their amino acids, serves to their function. Li et al (2005) reported the absence of carboxylesterases in human plasma or the possibility of finding them in negligible amounts, difficult to be detected by mass spectrometry techniques. They also reviewed their absence in monkey, pig, ruminant and chicken plasma.

Four families of carboxylesterases were reviewed by Satoh (2005) in various mammalian species, including *CES1, CES2, CES3* and *CES4,* the first two of them being extensively studied through their genetic variants in human, monkey, dog, mouse, rat and rabbit. Holmes et al (2010) reviewed five mammalian carboxylesterase gene families in human, mouse and rat, including the *CES5/Ces5* variant. An alignment of the previous variant names and the newly considered classification was performed, the previously named *CES6* or *CES8* and *CES7*, alias cauxin, being included as *CES4A* and *CES5A*. Considering *CES1*-*CES5A* genes, all of them are located in the chromosome 16, in two different clusters, one of them including *CES2, CES3,* and *CES4A.* 12 to 14 exons were reviewed for the mammalian *CES* genes (Holmes et al 2010). Five *CES* subfamilies were also reviewed by Satoh & Hosokawa (2006).

All previously mentioned carboxylesterases are involved in the process of hydrolytic metabolism of various compounds, in different sites of action, such as liver microsomes *(CES1, hCE-1),* intestine *(CES2, hCE-2, hiCE),* or in brain, liver and colon *(CES3)* (Holmes et al 2010). Bencharit et al (2003) reviewed the presence of *hCE-1 (also called egasyn),* not only in the liver, but also in the small intestine, kidney, lung, testes, heart, monocytes, macrophages and circulating plasma. *hCE-1* and *hCE-2* exhibit 48-49% of amino acid structure in their sequence identity and are predicted to be glycoproteins of ~62 kDa (Bosron & Hurley 2002; Redinbo & Potter 2005). Redinbo & Potter (2005) reviewed *hCE-3* to be expressed exclusively in the brain, sharing 77% and 49.5% of their sequence identity with *hCE-1* and *hCE-2*, respectively. *hCE-1* was identified as having a high sequence identity with several enzymes, such as *carboxylesterase form 1* in rabbit, liver proline-β-naphthylamidase in pig, hydrolase A in rat and carboxylesterase Es-22 in mouse (Pindel et al 1997).

Some of the catalytic activities of *hCE-1* and *hCE-2* on several pharmaceutical compounds were reviewed by Bosron & Hurley (2002), Pindel et al (1997), Redinbo & Potter (2005), Satoh (2005), Satoh & Hosokawa (2006). The specificity of *hCE-1* in the methylester of cocaine and of *hCE-2* in procaine, a benzoylester of cocaine, and the enzymatic conversion of 6-acetylmorphine to morphine were well documented. The benzoylester of cocaine is also metabolized by serum butyrylcholinesterase (which together with albumin hydrolyses aspirin – Li et al 2005) and both *hCE-1* and *hCE-2* are involved in the catalysis of heroin and irinotecan (the activation of the anticancer prodrug CPT-11 to its active metabolite SN-38), with a higher efficiency for *hCE-2* than for *hCE-1*, as also postulated Hatfield et al (2011). More specifically, Redinbo & Potter (2005) reviewed at least one more efficient activity of *hCE-2* compared to *hCE-1* in catalysing the conversion of CPT-11 to SN38. Interestingly, although *hCE-1* was reviewed by Loandos et al (2012) to be unable to process CPT-11 (7-ethyl-10-[4-(1-piperidino)-1-piperididino] carbonyloxycamptothecin), its homologous carboxylesterase in the rabbit's liver *(rCE)*, with 81% sequence identity, is considered the most efficient enzyme in this process of prodrug CTP-11 activation.

Associated human plasma cholinesterase - butyrylcholinesterase in cocaine and heroin metabolism. Pindel et al (1997) reviewed the presence of *hCE-2* in liver and the hydrolysis of benzoylester of cocaine by liver *hCE-2* and serum cholinesterase (butyrylcholinesterase, pseudocholinesterase, acylcholine acylhydrolase; EC 3.1.1.8), in order to produce ecgonine methyl ester and benzoic acid. The same products of catalysis

were reported as a result of cocaine hydrolysis by a bacterial cocaine esterase *(cocE)* produced by a strain of *Rhodococcus,* bacteria which is found out in the soil of cocaine-producing plants and whose enzyme seems to resemble to a substrate of specificity to *hCE-2* (Bosron & Hurley 2002). *hCE-1* is involved in the hydrolysis of the methyl ester of cocaine to benzoylecgonine and methanol. Cashman et al (1996) remarked benzoylecgonine as an inactive psychomotor stimulant comparable to the cocaine, and also that in the presence of ethanol, the transesterification of cocaine lead to cocaethylene and methanol, increasing therefore the toxicity of cocaine. The *hCE-1* involvment in the creation of the toxic metabolite cocaethylene from cocaine and alcohol, when they are abused together, was also discussed by Bencharit et al (2003), the resulted metabolite showing a longer half-life, a higher brain/plasma distribution ratio and a greater toxicity than cocaine. Earlier reports, such as that of Stewart et al (1977), also recognized the ability of the butyrylcholinesterase for cocaine hydrolysing, converting benzoylecgonine to ecgonine. Butyrylcholinesterase is synthesized in the liver (Krasowski et al 1997), it lacks in 1/3,000 individuals and it shows a higher sensitivity to the stimulant and toxic effects of cocaine, especially considering the prolonged half-time for its hydrolysis, from 30 minutes in plasma of normal individuals to more than two hours in plasma of those deficient, as Shuster (1990-1991) reviewed. In humans, the gene for butyrylcholinesterase is located on chromosome 3 and, although there is more than 50% homology of amino acid sequences, a similar intron-exon organization and number as in the acetylcholinesterase encoding gene, the latter differ in both nucleotide sequence and chromosomal location (human chromosome 7) (Krasowski et al 1997).

The metabolism of heroin occurs mainly at the liver level, involving both *hCE-2* and *hCE-1,* and also the serum cholinesterase - butyrylcholinesterase. As already mentioned, the higher efficiency is known for *hCE-2*; although all three enzymes cleave the 3-acetyl group of heroin to form 6-monoacetylmorphine, only *hCE-2* is able to further hydrolyse 6-monoacetylmorphine to morphine with a high catalytic efficiency (Bosron & Hurley 2002; Pindel et al 1997; Satoh & Hosokawa 2006).

Conclusions. Although homologies on rabbit chromosome 5 and human chromosome 16 were reported, different genes are involved in atropine and cocaine metabolism in these species. In rabbits, a specific atropinesterase locus *(Est-2)* is known to be linked with the locus for cocainesterase *(Est-1).* On the other hand, in cocaine metabolism, no specific atropinesterase activity was reported in humans, but only the three following enzymes: *hCE-1,* with a high sequence identity homology with rabbit liver carboxylesterase – *rCE*, *hCE-2,* and butyrylcholinesterase – a serum cholinesterase whose gene is located on a different chromosome than the previous two encoding genes in humans. These various aspects are important for the medical research, in particular regarding the topics in which the rabbit can be considered as human model.

Conflict of interest. The author declares no conflict of interest.

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Received: 03 May 2021. Accepted: 05 July 2021. Published online: 16 July 2021.
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How to cite this article:
Grădinaru A. C., 2021 Different drug-processing enzymes with atropine- and cocainesterase activities reported in rabbits and humans. Rabbit Gen 11(1):10-15.