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Accelerating cocaine metabolism as an approach to the treatment of cocaine abuse and toxicity

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Abstract

One pharmacokinetic approach to the treatment of cocaine abuse and toxicity involves the development of compounds that can be safely administered to humans and that accelerate the metabolism of cocaine to inactive components. Catalytic antibodies have been developed and shown to accelerate cocaine metabolism, but their catalytic efficiency for cocaine is relatively low. Mutations of human butyrylcholinesterase and a bacterial cocaine esterase found in the soil of coca plants have also been developed. These compounds accelerate cocaine metabolism and antagonize the behavioral and toxic effects of cocaine in animal models. Of these two approaches, the human butyrylcholinesterase mutants show the most immediate promise as they would not be expected to evoke an immune response in humans.

Cocaine use remains a major public health concern in many countries throughout the world. In the most recent survey of emergency departments in the USA, cocaine use was mentioned in over 45% of visits where illicit drug use was a factor [1]. Thus, there clearly remains a need for a treatment for cocaine abuse and for the effects of cocaine that may lead to an emergency department admission. In fact, considerable research effort has been focused on developing pharmacological treatments that target the receptor systems implicated in cocaine abuse [2–4]. This research effort has focused on a variety of receptor systems, but, despite this research effort, there is still no approved medication for the treatment of cocaine abuse. One of the difficulties in developing a pharmacodynamic (PD) treatment for cocaine use may be the broad range of neurochemical systems that cocaine interacts with. Cocaine blocks the reuptake of dopamine, serotonin, norepinephrine and epinephrine. In addition, cocaine is a potent sodium channel blocker. PD treatments that focus on only one of these neurochemical systems may not be fully effective in counteracting the effects of cocaine. Furthermore, it may be difficult to pharmacologically alter the effects of a pharmacological ‘blocker’. Therefore, avenues of treatment that do not focus on the neurochemical effects of cocaine may prove more promising as treatments.

One alternative to a neurochemical approach is to attack the cocaine molecule directly before it reaches its site of action within the central nervous system. One such approach under consideration is the development of a cocaine vaccine. Under this approach, cocaine is linked to an immunogenic carrier protein. Some of the antibodies that develop to this

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conjugate vaccine will be specific for the attached cocaine molecule. Fox *et al.* [5] used this approach to develop a cocaine vaccine and showed that it would block cocaine self-administration in rats [6,7]. A similar approach has been shown to produce cocaine antibodies in humans [8] and successfully reduce the subjective effects of cocaine in humans [9] and cocaine use by addicts in outpatient treatment [10]. Modifications of this approach are still under active preclinical development [11]. In addition to a cocaine vaccine, passive administration of anticocaine monoclonal antibodies has also been shown to reduce the effects of cocaine [12,13]. However, there is considerable variability in cocaine antibody development, even for the best candidate vaccines [14]. In addition, there are ethical concerns with the use of vaccines [15], as the detection of anticocaine antibodies would be an indication that someone was in treatment for cocaine abuse.

For both active and passive immunization, each molecule of the antibody binds to a single cocaine molecule. As this bound complex is too large to cross the blood–brain barrier, the effect of the treatment is to sequester the cocaine in the periphery and, thus, reduce cocaine's CNS effects. However, because of this one-to-one correspondence between antibody molecules and cocaine, it is necessary to maintain high enough levels of antibodies to bind most of the cocaine administered. In contrast, molecules that can metabolize cocaine can process multiple cocaine molecules quickly depending on the speed at which they break down cocaine.

It has also long been recognized that altered metabolism of cocaine can alter the behavioral and toxic effects of cocaine. Figure 1 summarizes the metabolism of cocaine in humans. Cocaine is metabolized by plasma butyrylcholinesterase (BChE) [16,17] to ecgonine methyl ester (EME). Cocaine is also metabolized to benzoylecgonine by tissue esterases and spontaneous conversion. In humans, there is only minimal metabolism to norcocaine via CYP450 in the liver. Thus, any change in the activity of BChE might be expected to change the levels of cocaine in the body. Carmona *et al.* showed that rhesus monkeys differ from squirrel monkeys in their endogenous levels of BChE and that these different levels of BChE were associated with differences in the speed of cocaine metabolism in plasma between the two species [18]. Thus, differences in BChE plasma levels can alter the blood levels of cocaine, which, in turn, could alter any effect related to body levels of cocaine. Cocaine use does seem to be associated with more adverse clinical outcomes in patients that have lower endogenous BChE levels [19–22].

Morishima *et al.* showed that male rats have more toxic 'manifestations' following cocaine use than female rats and that male rats have lower endogenous BChE levels than females [23]. In animals, inhibition of BChE by compounds such as organophosphate poisons can also increase the toxic effects of cocaine. For example, Hoffman *et al.* showed that treating mice with the organophosphate poison parathion prior to cocaine administration increased the lethal effects of cocaine in mice [24]. Duysen *et al.* recently reported that in knockout mice that do not produce BChE, increased hepatotoxicity and more cardiac fibrosis was observed following cocaine treatment than in wild-type mice [25]. Herschman and Aaron presented a case report of a woman who ingested an organophosphate insecticide in the hope of prolonging the effects of cocaine [26]. These reports clearly show that lowered levels of BChE in plasma can potentially increase effects of cocaine by decreasing the metabolism of cocaine. It is reasonable to assume that increasing the metabolism of cocaine may decrease cocaine's effects if the metabolites produced are not in themselves active. This pharmacokinetic approach to drug treatment is under active investigation.

BChE metabolizes cocaine to the cocaine metabolite EME [16,17]. Unlike cocaine, EME does not appear to have any CNS effects and may counteract some of the effects of cocaine [27]. Therefore, a treatment that accelerates the metabolism of cocaine through a mechanism

similar to BChE may be able to reduce the amount of cocaine that enters the brain and counteract the abuse-related effects of cocaine. Accelerating the metabolism of cocaine would also reduce the overall body load of cocaine and, therefore, might also reduce the toxic effects of cocaine, even those associated with peripheral mechanisms. Currently, there are three different areas of investigation that are focusing on this approach to treating cocaine abuse and toxicity. An early approach in this area was to develop catalytic antibodies to cocaine that also metabolize cocaine to EME [28]. While it has been difficult to develop catalytic antibodies with sufficient activity against cocaine to be useful in humans, this approach is still being pursued. The second approach initially investigated BChE itself as potential treatment and is the most direct [29]. However, since BChE is actually more effective at metabolizing (+)-cocaine rather than the abused form of (–)-cocaine [30], this area of research has recently focused on mutations of human BChE that are more effective against (–)-cocaine. Finally, a bacterial enzyme found in the soil of the coca plant was also found to metabolize cocaine to EME [31]. This compound (known as bacterial cocaine esterase [CocE]) as well as mutations of CocE are also being pursued as potential cocaine treatments.

Cocaine catalytic antibodies

To develop catalytic antibodies [32], haptens must be prepared that mimic the transition state desired. In this case the transition state is the intermediate molecule along the reaction pathway from cocaine to the metabolic products EME and benzoic acid. Transition state analogs (TSAs) are prepared that mimic this intermediate molecule and are linked to a carrier protein to elicit an immune response, usually in mice. The antibodies produced are screened for catalytic activity and those that show activity are prepared for use through standard monoclonal techniques. Landry *et al.* used this technique to prepare a monoclonal antibody (mAb) that was able to metabolize cocaine [28]. The mAb 3B9 metabolized cocaine with a K_m of 490 μM and a K_{cat} of 0.01 min^{-1} . In analyzing the usefulness of this mAb against cocaine *in vivo*, Landry and colleagues proposed that any treatment that metabolizes cocaine should hopefully have a K_m of less than 30 μM and a K_{cat} of more than 120 min^{-1} . While this first effort did not reach that level of activity, Landry *et al.* suggested that a compound with activity approaching this level might be useful against cocaine *in vivo*.

In a second attempt to develop a cocaine catalytic antibody, Landry *et al.* (Yang *et al.* [33]) developed additional TSAs and screened antibodies to these TSAs for catalytic activity. Two mAbs were developed that improved on the enzymatic activity of their original compound. The mAb 8G4G had a K_m of 530 μM and a K_{cat} of 0.6 min^{-1} . While a slight improvement over the mAb 3B9 in terms of enzyme efficiency, this compound still did not approach the level of activity Landry and colleagues suggested might be useful. The second mAb reported on had much better activity. The mAb 15A10 had a K_m of 220 μM and a K_{cat} of 2.3 min^{-1} . While still not at the level Landry *et al.* [28] suggested as necessary for use *in vivo*, Yang *et al.* suggested this compound's activity may be high enough to show some utility in altering cocaine's effects.

A number of investigators have used mAb 15A10 to show its potential utility in treating cocaine toxicity and abuse. Mets *et al.* reported on the effects of mAb 15A10 against cocaine *in vivo* in rats [34]. In rats pretreated with mAb 1510 before cocaine administration, levels of EME were increased suggesting that mAb 15A10 had enhanced cocaine metabolism. In rats that were self-administering cocaine, pre-treatment with mAb 15A10 was able to decrease self-administration responding to levels seen following saline substitution for cocaine. The half-life of the effect of mAb 15A10 was estimated to be less than 24 h, as the effect was completely gone in 48 h. Pretreatment with mAb 15A10 was specific to cocaine, as responding for milk or bupropion were not affected.

Baird *et al.* tested mAb 15A10 against a range of cocaine self-administration doses in rats and found that pretreatment decreased cocaine self-administration and shifted the dose-effect function to the right [35]. Again mAb 15A10 did not affect responding for a milk reward. The effect of mAb 15A10 was still evident 48 h after administration, but not by 72 h. Baird and colleagues suggested that the relatively short duration of action of mAb 15A10 was due to the fact that mAb 15A10 was a mouse antibody being used in rats [35]. To be fully effective in humans, the monoclonal antibody would have to be humanized. To test this hypothesis, Briscoe *et al.* studied the effects of mAb 15A10 in mice where the antibody was developed [36]. Mice were treated with a range of doses of mAb 15A10 prior to receiving 100-mg/kg cocaine. Blood pressure was measured following cocaine treatment. Cocaine alone increased blood pressure, an effect that was antagonized by mAb 15A10. The effect of mAb 15A10 was still significant 3 days following administration, and a small nonsignificant effect was observed 10 days following administration. At 30 days following administration, the effect of mAb 15A10 against cocaine-induced blood pressure increases was completely eliminated. While the catalytic activity of mAb 15A10 was not up to the standard Landry *et al.* originally proposed, it was still effective in reducing some of cocaine's effects and had a reasonable duration of action [28]. Nevertheless, a compound that had better catalytic activity and longer duration of action would be desirable.

Unfortunately, other attempts at developing a cocaine catalytic antibody have not been as successful as those of Landry and colleagues. These studies have used other TSAs, but, so far, the catalytic antibodies produced have had K_m values in the millimolar range and/or K_{cat} values well below 1 min^{-1} [37–40]. Attempts to mutate previously identified cocaine catalytic antibodies have not been any more successful [41]. One exception to this is the catalytic antibodies developed by Cashman *et al.* [42]. One of these antibodies had a K_m of $105 \mu\text{M}$ and a K_{cat} of 38 min^{-1} for (–)-cocaine. This antibody has not been tested *in vivo*.

BChE & derivatives

As noted above, perhaps the most direct approach to the pharmacokinetic treatment of cocaine abuse and toxicity is the use of BChE. BChE is an enzyme naturally found in humans that metabolizes cocaine to the inactive metabolite EME. Therefore, the administration of additional BChE may be able to speed the metabolism of cocaine sufficiently to decrease the amount of cocaine in the body and to reduce its entry into the brain. As a result, added BChE may potentially reduce the behavioral and toxic effects of cocaine.

The use of added BChE had been tested for safety prior to its application in cocaine use when it was proposed as a treatment for chemical weapons exposure. BChE was able to protect against the nerve agents soman and sarin. In animals treated with these agents, BChE protected against both the toxic and behavioral effects of these agents [43–46]. Furthermore, BChE had a relatively long duration of action of over 50 h [29] and appeared to be safe when given alone, at least when the form given was derived from that species. In addition, there have also been methods developed for the rapid and large-scale production of BChE necessary for the use of the compound in treatment [47,48].

The enzyme kinetics of BChE in metabolizing cocaine compare favorably to those of the catalytic antibodies (Table 1). The K_m value for BChE is below the standard that Landry *et al.* suggested would be necessary for a compound to be useful in treatment [28]. While the K_{cat} fails to reach the standard Landry *et al.* set, it is clearly improved over the catalytic antibodies and, thus, the catalytic efficiency (K_{cat}/K_m) is clearly improved. Mattes *et al.* used a human form of BChE to treat rats subsequently treated with a high dose of cocaine (179 mg/kg, intraperitoneally [ip.]) [49]. They found that BChE could reduce plasma and

brain concentrations of cocaine by over 80%. Similarly in cats, Mattes and colleagues showed that pretreatment with BChE prior to cocaine could alter cocaine metabolism, with increased levels of EME observed along with decreased levels of the active metabolite norcocaine [49]. Browne *et al.* [50] showed that human plasma spiked with BChE accelerated the metabolism of cocaine and Carmona *et al.* [51,52] showed that in rats treated with horse serum-derived BChE, the cocaine half-life was significantly reduced. Also using horse serum-derived BChE, Koetzner and Woods showed that cocaine plasma and brain levels in mice were decreased following treatment with the enzyme [53].

Treatment with BChE has also been shown to be effective against cocaine's toxic effects. Rats pretreated with a human form of BChE survived a high dose (179 mg/kg ip.) cocaine treatment, while rats not treated with BChE did not [49]. Lynch *et al.* [54] was able to show treatment with BChE post cocaine was also able to protect rats against cocaine's cardiovascular and lethal effects, an effect that would be necessary if BChE was to be used in an emergency room setting. In cats, BChE pretreatment antagonized cocaine's pressor effect. Hoffman *et al.* showed that purified human BChE given to mice was able to protect against the lethal effect of cocaine [24].

Since BChE can speed cocaine metabolism and protect against the toxic effects of cocaine, it is reasonable to assume that BChE may also be able to reverse some of the behavioral effects of cocaine. Mattes *et al.* gave rats human BChE prior to treatment with a locomotor-activating dose of cocaine and found that BChE reduced activity compared with rats treated with cocaine alone [49]. Likewise, Carmona *et al.* [51] and Koetzner and Woods [53] showed that horse serum-derived BChE could antagonize the locomotor activating effects of cocaine. Figure 2 shows the results from Carmona and co-workers illustrating this effect. Rats were treated with BChE (5000 units) or saline and placed in an activity monitor for 30 min (−30 to −10). The rats initially show high levels of activity, but quickly habituate to the chamber as shown by the decrease in activity over the course of the 30 min habituation period. BChE did not affect habituation. Rats were then treated with 17-mg/kg cocaine or saline ip. Those rats given cocaine following saline show an increase in distance traveled. BChE clearly antagonized this effect, although some increase in activity above saline treated rats was observed. Those animals treated with BChE before saline treatment were comparable to saline–saline-treated animals, showing that BChE itself did not affect activity. Interestingly, there have been no published results investigating the effects of BChE on cocaine self-administration. While not published, our lab treated rats with horse serum-derived BChE prior to cocaine self-administration on a fixed-ratio schedule [GN Carmona, CW Schindler, Unpublished Data]. In the few rats tested, no effect of BChE was observed. This result suggests that the kinetic efficiency of BChE against cocaine may not be sufficient to alter an effect like cocaine self-administration that is dependent on the rapid uptake of cocaine into the brain. As a result, a number of investigators have investigated alterations of BChE in an attempt to increase its ability to metabolize cocaine.

Table 1 lists mutations of human BChE according to their amino acid substitutions. By noting that BChE was able to metabolize (+)-cocaine more efficiently than (−)-cocaine, investigators were able to focus on mutations to improve the ability of BChE to bind (−)-cocaine. Even a single amino acid substitution was able to increase the catalytic efficiency of BChE. The A328W mutant of BChE had a K_{cat} over three-times that of wild-type BChE and a relatively long half-life of 16 h [55]. In mice treated with A328W prior to cocaine, the locomotor-activating effects of cocaine were attenuated. Additional mutations of BChE proved to be even more efficient. The double mutant A199S/A328W had a K_{cat} for cocaine of 173 min^{-1} [56], clearly exceeding the standards suggested by Landry *et al.* [28]. The double mutant A328W/Y332A [57] also clearly exceeds that standard. This mutant, and some other BChE mutants, are sometimes referred to as CocE, which should not be

confused with the bacterial CocE described in the next section. The addition of this mutant to plasma or pretreatment with A328W/Y332A accelerated cocaine metabolism and decreased the cocaine half-life in rats [57,58]. Gao and Brimijoin showed that pretreatment with this mutant was able to blunt the cardiovascular effects of cocaine. They determined the half-life of the A328W/Y332A mutant to be 10 h [59]. Treatment with this mutant was also able to completely block the locomotor-activating effect of cocaine [57].

The triple mutant A199S/F227A/A328W [56] and the quadruple mutant F227A/S287G/A328W/Y332M [60] further improved on the catalytic efficiency of BChE for cocaine (Table 1). The quadruple mutant is also referred to as AME359 [61] and was shown by Gao *et al.* [62] to decrease cocaine plasma levels and to antagonize the cardiovascular effects of cocaine. Another quadruple mutant of BChE (A199S/F227A/A328W/Y332G) has proven to be even more efficient in catalyzing cocaine [60,63]. This mutant is often referred to as CocH and is typically fused to the C terminus of human serum albumin (Albu-CocH) to increase the peripheral circulation half-life for use *in vivo*. This compound has been studied extensively in animals.

When rats and squirrel monkeys are pre-treated with Albu-CocH, cocaine plasma levels are reduced and EME levels are increased [64,65]. Albu-CocH is also able to reduce cocaine brain levels [66]. This reduction in cocaine plasma levels is associated with a reduction in the toxic effects of cocaine. When rats were pretreated with Albu-CocH, cocaine-induced seizures were reduced [66], as shown in Figure 3. In this study, rats were treated with 10-mg/kg Albu-CocH and 10 min later were given cocaine. Seizure activity was then monitored. Pretreatment with Albu-CocH produced a substantial and significant shift to the right in the dose effect function for seizures. This effect of Albu-CocH was also seen when the mutant was delivered after, rather than before, cocaine administration, an important finding given that individuals would necessarily need to be treated after cocaine administration in an emergency room setting. Albu-CocH was also able to blunt the hypertensive effect of cocaine when administered in rats prior to cocaine [66]. These results suggest that Albu-CocH might be useful in emergency room settings to treat cocaine toxicity.

To be potentially useful as a treatment for cocaine addiction, Albu-CocH should also be able to reduce some of the abuse-related behaviors associated with cocaine administration. Albu-CocH has been shown to antagonize cocaine self-administration in rats responding on a progressive ratio schedule [67] and in squirrel monkeys responding on a fixed-ratio schedule [65]. In both rats and squirrel monkeys, Albu-CocH was also able to decrease cocaine-induced reinstatement of cocaine self-administration that had undergone extinction [65,66]. Finally, in squirrel monkeys, Albu-CocH is able to antagonize the discriminative stimulus effects of cocaine [65]. In the effects on squirrel monkey self-administration, Albu-CocH had a duration of action of over 24 h. After 24 h these effects were diminished. In monkeys, some evidence of immunogenicity was also observed. However, since Albu-CocH is derived from human BChE these effects might be less likely in humans.

A mutant with five amino acid substitutions (A199S/F227A/S287G/A328W/E441D) has also shown favorable enzyme kinetics against cocaine [56,68]. This compound has been shown to be effective against cocaine-induced seizures and to protect against the lethal effects of cocaine [68].

Research on BChE mutants has also focused on extending the presence of these mutants in the body. One technique that has been used successfully is virally mediated gene transfer. Gao *et al.* used this technique with the A328W/Y332A mutant [62]. They found that cocaine-hydrolyzing activity was increased 3000-fold and half-life of the mutant in plasma

was 33 h. When this technique was applied to the AME359 mutant, cocaine plasma-hydrolyzing activity was increased 50,000-fold. Cocaine was cleared so rapidly from the body that it was reduced by over 95% at the first-time sample (4 min). This treatment also antagonized the pressor effects of cocaine. In a subsequent study, Gao and Brimijoin injected these vectors directly into the nucleus accumbens and showed a dramatic increase in BChE activity as a result of those injections [69]. Gao and Brimijoin showed that when treated with the gene-transferred quadruple mutant intravenously, plasma BChE activity was substantially enhanced for up to 7 days and the ability of cocaine to induce c-Fos expression in the caudate nucleus was reduced for up to 7 days [70]. These results show that gene transfer techniques can be applied to the BChE mutants to substantially increase their duration of action, making them viable candidates for the treatment of cocaine abuse. A similar approach has been applied to BChE itself, but this compound has only been tested against the toxicity of chemical nerve agents or organophosphate poisons [71].

Bacterial cocaine esterase

Bressler *et al.* reasoned that there may be a bacterium in soil samples taken from around coca plants (the natural source of cocaine) that would be capable of metabolizing cocaine to use it as a source of carbon for growth [31]. They isolated a bacterium that they termed MB1 that was able to hydrolyze cocaine to EME and benzoate, similarly to BChE. Subsequently, this bacterial CocE has come to be known as CocE. Bressler and colleagues found that this bacterium was capable of metabolizing cocaine with a K_m of 1.3 mM. Subsequent investigators [72–74] have reported a much lower K_m for CocE and a K_{cat} of several hundred per min (Table 2), well within the standards set out by Landry *et al.* [28] as useful for treatment. Thus, this bacterial CocE has kinetic activity for metabolizing cocaine that makes it a suitable candidate as a treatment for cocaine toxicity.

Cooper *et al.* showed that in human plasma spiked with CocE, cocaine metabolism was accelerated. CocE given to rats was also able to antagonize cocaine lethality, but not that of WIN 35,065-2, a compound with a different structure but similar activity to cocaine [72]. This result suggests that CocE's effects were primarily related to its ability to metabolize cocaine. CocE given 100 min prior to cocaine was not able to antagonize the lethal effects of cocaine and Cooper and co-workers determined that the half-life of CocE was 13 min. Ko *et al.* confirmed CocE's effectiveness against cocaine lethality, and also showed that as little as a 20-min pre-treatment time would reduce CocE effectiveness and also showed that repeated treatment with CocE reduced its effectiveness, suggesting that antibodies were being developed to CocE [75]. Ko *et al.* [76] were able to show that antibodies to CocE were indeed observed following repeated treatment. Jutkiewicz *et al.* [77] and Wood and colleagues [78] were able to show that the effect of CocE against cocaine toxicity extended to the convulsive and cardiovascular effects of cocaine. However, with such a short half-life, CocE would not be useful against the behavioral effects of cocaine. Furthermore, its ability to elicit an antibody response would also reduce its effectiveness as a drug abuse treatment where multiple administrations would be required.

Taking the same approach used with BChE, investigators have recently mutated CocE in an attempt to extend its half-life and improve its catalytic efficiency. Narasimhan *et al.* reported on two CocE mutants (L169K and T172R/G173Q) that not only improved on the catalytic efficiency of CocE (Table 2), but also extended the half-life of the esterases [79]. The L169K mutant had a half-life of 570 min, while the double mutant, T172R/G173Q, had a half-life of 370 min. The double mutant has been tested for activity against cocaine's toxic effects. Collins *et al.* [80,81] showed that T172R/G173Q could antagonize cocaine-induced lethality and seizures in rats, and later showed that it could block the cardiovascular effects of cocaine in rhesus monkeys [82] and rats [81]. While Collins and co-workers did report

that CocE antibodies were detected in rhesus monkeys, repeated administration of the T172R/G173Q double mutant did not seem to diminish its effects [82]. The longer half-life of the double mutant also made it a suitable candidate for testing against the behavioral effects of cocaine. Collins *et al.* reported that the T172R/G173Q mutant was able to antagonize cocaine self-administration in the rat, with an apparent shift to the right in the dose–effect function [80]. Figure 4 shows the effects of four doses of the double mutant CocE given as a pretreatment before cocaine self-administration sessions in rats. The highest dose of the double mutant CocE was able to reduce self-administration of 0.1-mg/kg/injection cocaine to saline levels.

Recently, another double mutant of CocE, T169K/G173Q, has been described [83]. The T169K/G173Q mutant was reported to have a half-life of 2.9 days and was able to block both the lethal effects of cocaine in mice and cocaine self-administration in rats. Brim *et al.* also reported on other mutants of CocE, but none had the catalytic efficiency of the T169K/G173Q double mutant. Thus, the mutation of CocE can greatly extend its catalytic efficiency and duration of action and, thus, makes it a more viable candidate for treating cocaine abuse. A longer duration of action would not be as critical in treating cocaine toxicity where any treatment would need to immediately reverse the effects of cocaine, but where additional cocaine use would not be anticipated in the immediate future. However, since this is an enzyme derived from soil bacteria, antibody development would be a concern when the compound is given to humans.

Future perspective

Table 3 summarizes the research findings with the three most promising candidate medications for the catalytic antibody, mutant BChE and mutant CocE approaches described above. While the catalytic antibody approach has seen some success in preclinical research, the catalytic activity of the best compound from this series is still relatively weak in comparison to the other approaches. When matched to the species tested, however, this approach does have a reasonable duration of action. In contrast, the Albu-CocH mutant of BChE has impressive catalytic activity and preclinical research has shown it to be effective against both cocaine toxicity and cocaine abuse-related behaviors. While this compound's duration of action may be over 24 h, any increase in the duration of action would be a significant improvement. A gene therapy approach that has been applied to two of these BChE mutants also shows promise of extending the duration of action for these compounds [62,69,70]. We are not aware of any of these compounds having been tested in humans for safety, which would be a necessary first step. However, since these compounds have been derived from human BChE with only minimal amino acid substitutions, they would not be expected to produce any untoward effects in humans. Nevertheless, since these compounds are different than endogenous BChE, evaluation of immunogenicity would still be necessary. Research using BChE to counteract the effects of nerve agents supports this assumption [84]. Therefore, the use of mutated forms of human BChE shows great promise in the treatment of cocaine abuse and toxicity and will likely be tested in humans in the near future.

Like the approach used with BChE, mutants of CocE have been developed that have much longer durations of action. The T172R/G173Q mutant has impressive catalytic activity and has also been shown to be effective against some of the behavioral effects of cocaine related to abuse in animal models. However, because this compound was developed from a soil bacterium, it is likely that it would evoke an immune response when given to humans or animals. In fact, evidence of immune responses has been observed in animals [82]. A modification of this compound that reduces its immune response would appear to be a necessary prelude to its use in humans [85]. The *in vivo* duration of action of this compound

could also to be improved. Recently, the use of a PEGylated form of this mutant CocE was tested against both the reinforcing and discriminative stimulus effects of cocaine and duration of action of up to 72 h was observed [86]. Thus, like Abu-CocH, the double mutant form of CocE holds great promise as a candidate treatment medication for both cocaine toxicity and abuse.

It is important to note that each of these approaches has been proposed for use in emergency rooms for the treatment of acute cocaine toxicity. While it is likely that all would be effective, since a short duration of action would not be drawback to use in this setting, their overall utility may be limited. To begin with, cocaine is a short duration drug. Thus, an individual experiencing an acute toxic reaction to cocaine would have to present for emergency care soon after administration. Furthermore, the action of these approaches is very specific for cocaine. Thus, BChE mutants would not be expected to metabolize other compounds unless they are also metabolized by endogenous BChE. The same holds true for the CocE mutants [87]. Thus, emergency care providers would need to recognize that the patient was presenting with cocaine-related toxicity, as this approach would not be effective against other drugs. Since the toxicity associated with other psychomotor stimulants such as amphetamine and methamphetamine would likely mimic those of cocaine, this would need to be done quickly. Nevertheless, this approach may be useful under a specific set of circumstances when a patient presents for emergency care where they are known to have used cocaine.

As noted above, much of the research on pharmacotherapies for cocaine abuse focuses on a variety of receptor systems in the brain that are thought to be involved in the reward process. While much of the early work in this area focused on dopamine, recently this work has expanded to include a variety of other receptor systems. One advantage of targeting the brain-reward system is that any potential treatment may be equally effective against other drugs of abuse. That is, a drug that targets the reward system in such a way as to disrupt cocaine relapse, may also disrupt relapse for other drugs of abuse such as amphetamine. In contrast, accelerating cocaine metabolism through the use of a mutant BChE compound would be restricted to cocaine only. However, any treatment drug targeting the brain reward system might also affect behavior unrelated to drug abuse to the degree that the reward systems for the two behaviors overlap. For example, while dopamine antagonists can reduce self-administration in rodents, slightly larger doses can also reduce operant responding for food [88]. Since a mutant BChE would be restricted to the periphery, it would be unlikely to affect other behaviors and in fact does not seem to affect food reinforced behavior [65]. Clearly, the side-effect profiles of the treatments reviewed here, as well as PD treatments, will be important considerations in their eventual use in humans.

In the next 5 years, it is likely that there will be preclinical refinements to each of these three approaches. The focus for the catalytic antibody approach will most likely be to improve its catalytic efficiency. The catalytic efficiency of both the human BChE and bacterial CocE approaches is clearly sufficient to make them useful as treatments for cocaine abuse and toxicity. Continued focus for both approaches will be on extending the duration of action of the compounds. For bacterial CocE, it will be necessary to evaluate the immune response to this compound and to determine what effects it might have in humans and whether the immune response can be limited or reduced. It is likely that we will see some testing in humans, particularly for the human BChE mutants, in the near future. Drugs that accelerate the metabolism of cocaine hold great promise for the treatment of cocaine abuse, at the very least as an adjunct to other behavioral or pharmacological, or peripheral (i.e., cocaine vaccines [89]), approaches to cocaine abuse treatment.

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Key Terms

Butyrylcholinesterase	Primary enzyme responsible for the metabolism of cocaine in humans
Catalytic antibody	Antibody developed with the purpose of metabolizing of a drug

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Executive summary

- One pharmacokinetic approach to treating cocaine abuse and toxicity seeks to develop drugs that accelerate the metabolism of cocaine to inactive metabolites in sufficient amounts to reduce the behavioral and toxic effects of cocaine.
- Catalytic antibodies have been developed for cocaine, but these antibodies are not very efficient in metabolizing cocaine.
- Mutations of human butyrylcholinesterase have been developed that are very efficient in metabolizing cocaine, have relatively long durations of action and are effective in a number of preclinical models of cocaine abuse and toxicity.
- A bacterial cocaine esterase has also been isolated and mutations of this compound are also very efficient in metabolizing cocaine, have relatively long durations of action and are effective in a number of preclinical models of cocaine abuse and toxicity. However, immune responses to these compounds have been detected in animals, potentially limiting their use in humans.
- Over the next 5 years it is likely that improvements to each of these approaches will be made. At this time the compounds that are derived from human butyrylcholinesterase and the bacterial cocaine esterase would appear most likely to be tested in humans.

Figure 1. Pathways for cocaine metabolism

Metabolism of cocaine occurs primarily via butyrylcholinesterase to ecgonine methyl ester or to benzoylecgonine via tissue esterases and spontaneous conversion. There is only minimal metabolism to norcocaine in humans.

Figure 2. Effects of 5000 IU horse serum-derived butyrylcholinesterase administered intravenously on cocaine-induced locomotor activity

Rats were treated intravenously with saline or butyrylcholinesterase just prior to being placed in the locomotor activity chamber. After a 30-min habituation period, animals were removed from the chamber and injected intraperitoneally with 17-mg/kg cocaine or saline and immediately placed back in the activity chamber.

BChE: Butyrylcholinesterase; COC: Cocaine; SAL: Saline.

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Figure 3. Pretreatment with 10-mg/kg of quadruple mutant of human butyrylcholinesterase (A199S/F227A/A328W/Y332G) shifts the dose-effect function for cocaine-induced seizures to the right in rats

The quadruple mutant of human butyrylcholinesterase was given 10 min prior to cocaine and the values beside the figures represent the number of rats per group.

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Figure 4. Effects of the double mutant cocaine esterase (T172R/G173Q) on cocaine self-administration

Rats responded for 0.1 mg/kg/injection cocaine during baseline sessions. The double mutant cocaine esterase was given before the start of the 60-min cocaine self-administration sessions. The higher doses of double mutant cocaine esterase reduced cocaine self-administration.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

CocE: Bacterial cocaine esterase; DM: Double mutant; PT: Pretreatment.

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Table 1

Butyrylcholinesterase and derivatives.

Compound	K _m (μM)	K _{cat} (min ⁻¹)	Cocaine metabolism	Toxicity	Cocaine-induced behavior
BChE	3–14 [60,90,91]	3.9–4 [60,90,91]	↓ cocaine plasma levels [49,53] ↓ cocaine brain levels [49] ↓ cocaine half-life [92] ↑ EME plasma levels [49,52,92]	↓ lethality [24,49,54] ↓ convulsion/seizures [24] ↓ cardiovascular [49,54]	↓ locomotor activity [49,51,53]
A328W [55]	10	30			↓ locomotor activity
A199S/A328W [56]	1.9	173			
A328W/Y332A (CocE)	18 [57]	154 [57]	↓ cocaine plasma levels [57,62] ↓ cocaine half-life [58]	↓ cardiovascular [59,62]	↓ locomotor activity [57]
A199S/F227A/A328W [56]	1.5	472			
F227A/S287G/A328W/Y332M(AME359)	20 [60]	620 [60]	↓ cocaine plasma levels [62]	↓ cardiovascular [62]	
A199S/F227A/A328W/Y332G (CocH)	2.1–3.1 [60,63]	2700–3600 [60,63]	↓ cocaine plasma levels [64,65] ↓ cocaine brain levels [66] ↑ EME plasma levels [65]	↓ convulsion/seizures [66] ↓ cardiovascular [66]	↓ self-administration [65,67] ↓ reinstatement [65,66] ↓ drug discrimination [65]
A199S/F227A/S287G/A328W/E441D	0.8–1.1 [56,68]	1326–1730 [56,68]		↓ lethality [68], ↓ convulsion/seizures [68]	

BChE: Butyrylcholinesterase; CocE: Cocaine esterase; CocH: Cocaine hydrolase; EME: Ecgonine methyl ester.

Table 2

Bacterial cocaine esterase and derivatives.

Compound	K_m (μM)	K_{cat} (min^{-1})	Cocaine metabolism	Toxicity	Cocaine-induced behavior
CocE	0.6–1.6 [72–74]	468 [72–74]	↓ cocaine plasma levels [72]	↓ lethality [72,75,76] ↓ convulsion/seizures [77,78] ↓ cardiovascular [78]	
L169K [79]	44	4806			
T172R/G173Q	17 [79]	3204 [79]		↓ lethality [80,81] ↓ convulsion/seizures [80,81] ↓ cardiovascular [81,82]	↓ self-administration [80,81]
T169K/G173Q [83]	58.3	12760		↓ lethality	↓ self-administration

CocE: Cocaine esterase.

Summary of effects for the most promising candidate medication for the catalytic antibody (monoclonal antibody 1510), mutant butyrylcholinesterase derivative (Albu-CocH) and mutant cocaine esterase derivative (T172R/G173Q) approaches.

Table 3

Compound	K _m (μM)	K _{cat} (min ⁻¹)	<i>In vivo</i> duration of action	Cocaine metabolism <i>in vivo</i>	Cocaine toxicity	Cocaine-induced behavior	Tested in primates?
Monoclonal antibody 1510	220	2.3	≤3 days	↑ EME plasma levels	↓ cardiovascular	↓ self-administration	No
Albu-CocH	2.1–3.1	2700–3600	>24 h	↓ cocaine plasma levels ↓ cocaine brain levels ↑ EME plasma levels	↓ convulsion/seizures ↓ cardiovascular	↓ self-administration ↓ reinstatement ↓ drug discrimination	Yes (behavior)
T172R/G173Q	17	3204	<24 h		↓ lethality ↓ convulsion/seizures ↓ cardiovascular	↓ self-administration	Yes (toxicity)

EME: Ecgonine methyl ester.