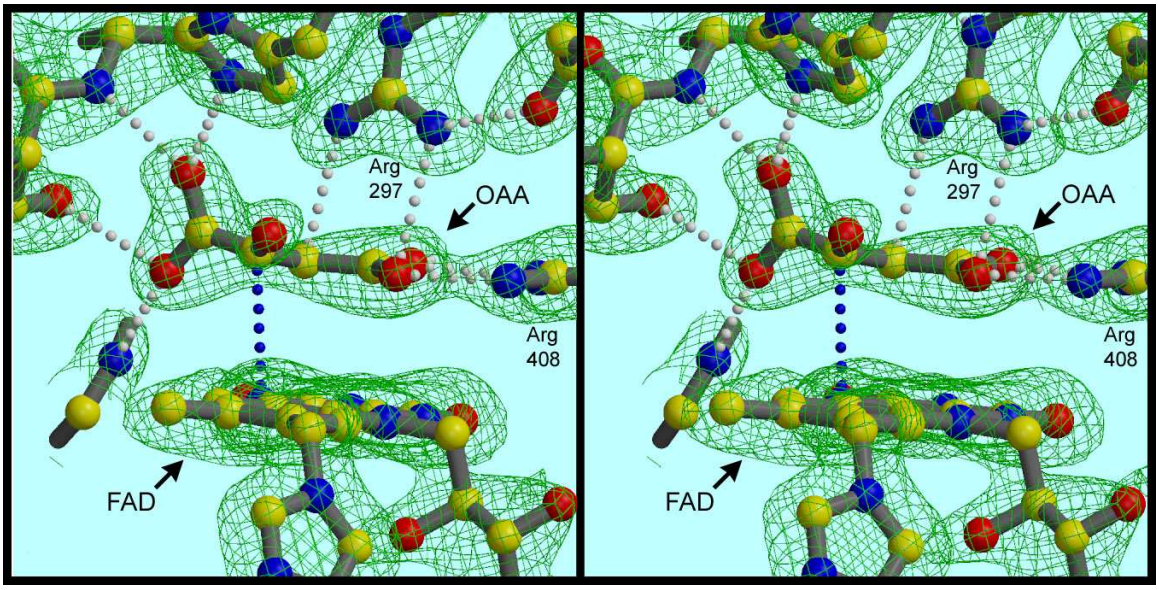



**Figure 12. Co-purified oxaloacetate binding in Complex II.**
The FAD flavin group lies flat at the bottom, viewed edge-on from the end attached to His63. Attachment of the ribitol chain is also visible. OAA lies flat on the flavin moiety, presumably much as the substrate would, strapped in by 8 hydrogen bonds (7 visible here) to the four carboxylate oxygens. Arg297 also makes an unusual H-bond to the C2 carbon (i.e. the atoms are closer than van-der-waals contact). The blue dotted line indicates the path of the postulated hydride transfer from C3 to N5 of the flavin (mechanism described in next slide). Note OAA is not planar, perhaps indicating the keto carbon no longer has SP2 geometry.

 From Lancaster et al. Eur. J. Biochem. 268, 1820-1827)
(mechanism of Chapman's group)

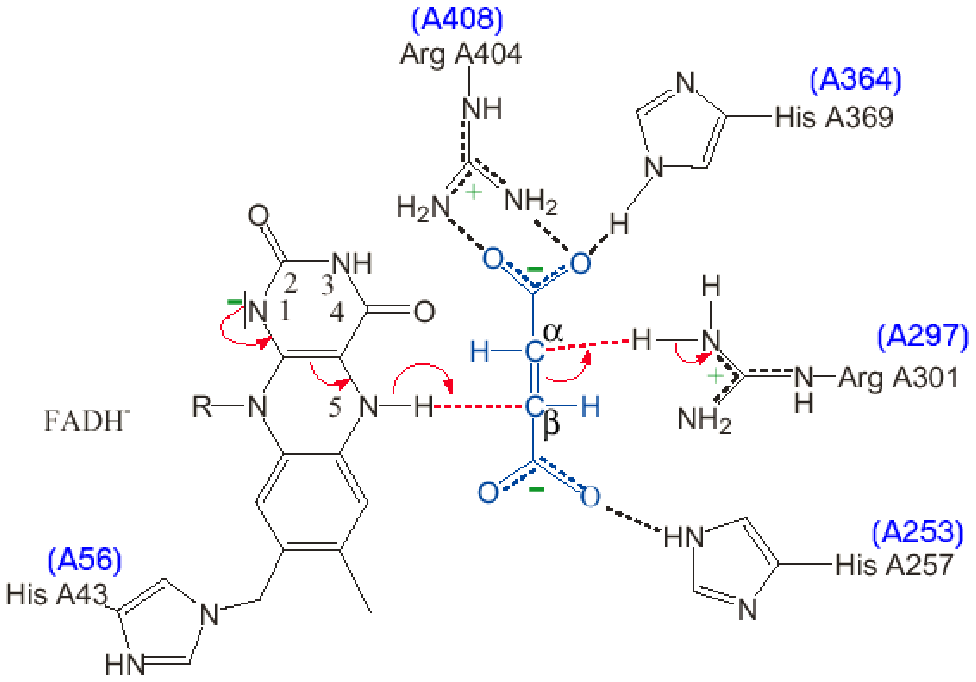



**Fig. 3. Possible mechanism of fumarate reduction in *W. succinogenes* QFR involving the residues shown in Fig. 2.** Hydride transfer from the N5 of FAD to the β-methenyl of fumarate (in blue) is coupled to proton transfer to the α position of the substrate from the side chain of Arg A301.

**Figure 13.**

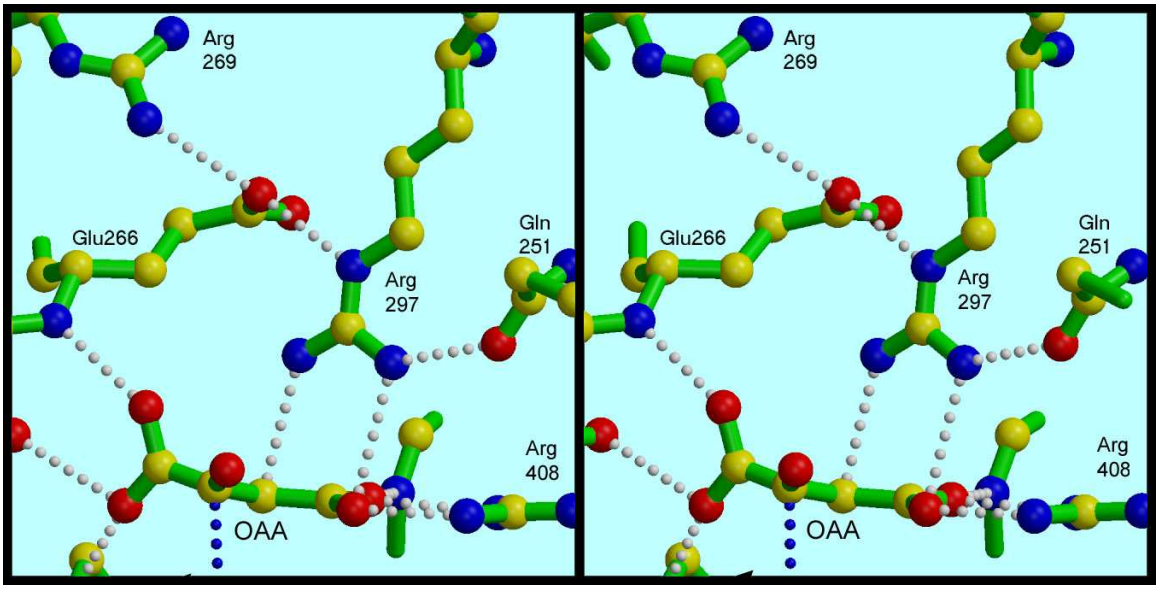



**Figure 14**. Arg297 is extremely well ordered, being covered in density by the 2Fo-Fc map contoured at 2 sigma. Presumably this is due to H-bonds to OAA, Gln251, and Glu266. The H-bonds to the negatively charged substrate carboxylate and Glu266 presumably enhance the basicity making it an effective catalytic base to abstract the proton.

Although this is similar to the picture in the flavocytochrome c substrate binding site, it has never been clearly shown in any of the membrane-bound fumarate reductases or SDH. This is may be because the residues of the site come from two different domains, and the available structures show some relative movement of the domains which disrupts the binding site in the high-resolution Wolinella structures or the E. coli FRD. The E. coli SDH structure (1nek) has nearly the same position of the "CAP" and "FAD" domains, but the equivalent of Arg297 is in a different rotamer. The same area of 1nek is superimposed with our structure in Figure x.

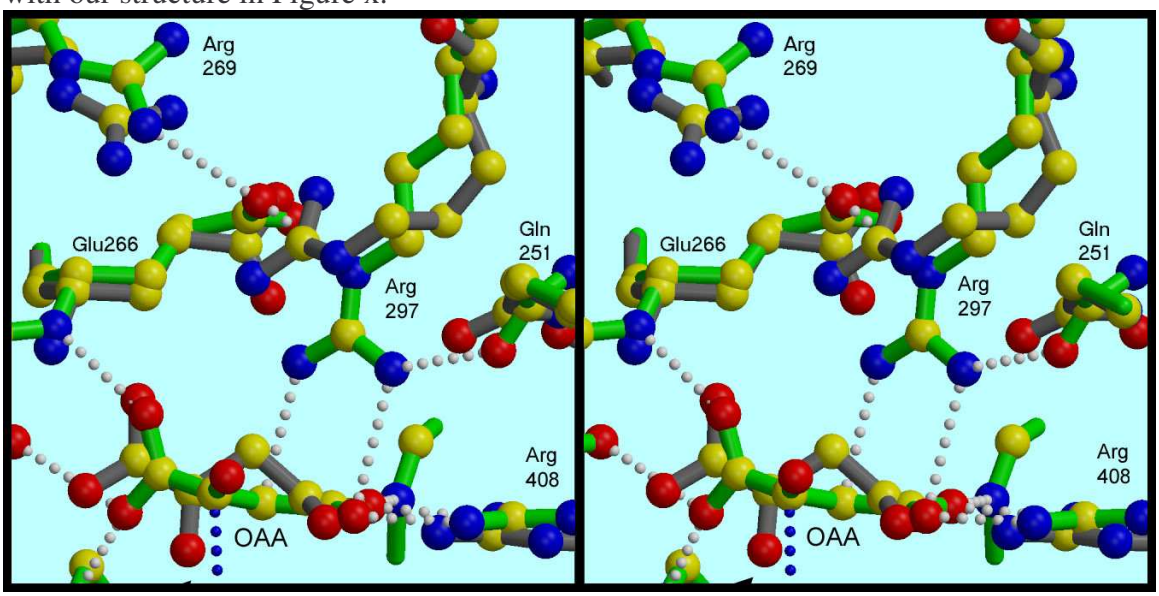
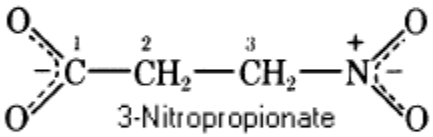
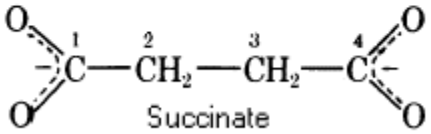



**Figure 15.** superposition of residues around the substrate binding site in structures 1NEK and the current. Arg297 makes no H-bonds with substrate of Gln251 in the 1nek conformation.

# 3-nitropropionic acid:

- A specific, irreversible complex II inhibitor
- Effective in vivo
- even when administered orally




1: Biomed Environ Sci. 1992 Jun;5(2):161-77. Related Articles, Links

**Studies on the epidemiology and etiology of moldy sugarcane poisoning in China.**

**Liu X, Luo X, Hu W.**

Institute of Nutrition and Food Hygiene, Chinese Academy of Preventive Medicine, Beijing.

Moldy sugarcane poisoning, an acute fatal food poisoning of unknown etiology, has occurred in 13 provinces in China. The epidemiological characteristics and clinical features were described. Evidence from laboratory studies indicates that 3-nitropropionic acid produced by the fungus Arthrinium Spp. is the etiological factor of this food poisoning.

PMID: 1642790 [PubMed - indexed for MEDLINE]

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1: J Toxicol Clin Toxicol. 1995;33(4):363-7. Related Articles, Links

**Moldy sugarcane poisoning--a case report with a brief review.**

**Ming L.**

Department of Pediatrics, First Hospital, Beijing Medical University, China.

A five-year-old girl developed an acute encephalopathy after eating a piece of moldy sugarcane. Delayed symptomatic dystonia was the main effect; cranial CT scans revealed bilateral lenticular lucencies. This case is typical of moldy sugarcane poisoning cases previously reported only in China. 3-Nitropropionic acid produced by Arthrinium sp is the most likely etiologic agent.

Publication Types:
- Case Reports
- Review
- Review of Reported Cases

PMID: 7629905 [PubMed - indexed for MEDLINE]

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However the effectiveness in vitro makes 3-NPA very useful for researchers who want to diminish complex II activity in a tissue or organism:

ELSEVIER

Brain Research Protocols 1 (1997) 253–257

BRAIN RESEARCH PROTOCOLS

Protocol

# Hyperactivity and hypoactivity in a rat model of Huntington's disease: the systemic 3-nitropropionic acid model

Cesario V. Borlongan *, Theodore K. Koutouzis, Thomas B. Freeman, Robert A. Hauser, David W. Cahill, Paul R. Sanberg

*Division of Neurological Surgery and the Neuroscience Program, Departments of Surgery, Neurology, Psychiatry, and Pharmacology, University of South Florida College of Medicine, 12901 Bruce B. Downs Blvd., MDC 16, Tampa, FL 33612, USA*

Accepted 2 May 1996

**Abstract**

The present study proposes the use of systemic 3-nitropropionic acid (3-NP) treatment in rats as a model of Huntington's disease (HD). The systemic 3-NP model involves chronic injection of low dose intraperitoneal (i.p.) injections of 3-NP to rats once every 4 days over a period of time. Evidence from our experimental studies suggests that manipulating the number of injections can result in either increased nocturnal spontaneous locomotor activity (hyperactivity) or nocturnal akinesia (hypoactivity) [1]. For example, two injections of 3-NP (using the treatment of one injection every 4 days) result in hyperactivity, while four injections or more of 3-NP lead to hypoactivity [1]. The locomotor activity is recorded by Digiscan locomotor activity monitors [11]. The observation of these two types of locomotor activity is unique since no excitotoxin model has replicated a two-stage progression of a HD-like behavioral alteration. Most studies using excitotoxins like quinolinic acid (QA) and kainic acid (KA) have only reproduced the hyperactivity stage [4,5,7]. With the systemic 3-NP model, investigations into at least two stages of the disease are made possible. This allows for better assessment of intervention strategies such as neural transplants across different stages of the disease. The systemic 3-NP rat model is believed to be an improved animal model of HD. © 1997 Elsevier Science B.V.

*Themes:* Disorders of the nervous system



Polish Journal of Pharmacology
Pol. J. Pharmacol., 2000, 52, 55-57
ISSN 1230-6002

## SEIZURES EVOKED BY MITOCHONDRIAL TOXIN, 3-NITROPROPIONIC ACID: NEW MECHANISM OF EPILEPTOGENESIS?

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2 Department of Clinical Toxicology, Institute of Agricultural Medicine, Jaczewskiego 2, PL 20-950 Lublin, Poland

*Seizures evoked by mitochondrial toxin, 3-nitropropionic acid: new mechanism of epileptogenesis?* E. M. URBANSKA. Pol. J. Pharmacol., 2000, 52, 55-57.

A number of data concerning the central action of mitochondrial toxins, substances impairing mitochondrial synthesis of ATP and thus compromising cellular energy status, has emerged within last years. 3-Nitropropionic acid (3-NPA) is an irreversible inhibitor of succinate dehydrogenase and mitochondrial complex II. The experimental administration of 3-NPA may lead to selective neuronal loss and chorea-like behavioral alterations but, as was recently shown, it also evokes clonic convulsions in rodents. The gathered data suggest that disturbed mitochondrial energy metabolism might initiate the chain of events culminating in seizure episode and that 3-NPA might become a useful tool in studying "mitochondrial" seizures. It has been hypothesized that the resistance to standard anticonvulsive therapy occurring among high proportion of epilepsy sufferers may result from the impairment of mitochondrial energy status due to either genetic predispositions or environmental influences.

***Key words:*** *mitochondrial toxins, 3-nitropropionic acid, seizures, epilepsy*

Back to: Top PJP - Home Page

1: Acta Neurochir Suppl. 2004;89:63-6. 

# Pharmacological preconditioning in global cerebral ischemia.

**Brambrink AM, Noga H, Astheimer A, Heimann A, Kempski O.**

Institut of Neurosurgical Pathophysiology, Johannes Gutenberg-University, Mainz, Germany.

Single dose 3-nitropropionic acid (3-NPA) 24 hr before global ischemia improves neuronal survival in both, neocortex and hippocampus ('chemical preconditioning'). Neuronal survival after transient global ischemia requires new protein synthesis during recovery, especially of those with anti-apoptotic function. Bcl-2-protein is expressed in neurons that survive cerebral ischemia and may parallel the time course of tolerance after ischemic preconditioning. With this study we examined whether differences in bcl-2-protein expression compared to baseline may be involved in the induction of ischemic tolerance using 3-NPA. Male Wistar rats received either a single intraperitoneal (i.p.) dose of 3-NPA (20 mg/kg), and were observed for 3 (n = 4), 12 (n = 5) or 24 hours (n = 5) or the same amount of vehicle and were observed for 24 h (n = 8, controls). Immunohistochemistry allowed to compare the intensity of bcl-2 immunoreactivity at three subsequent time points in hippocampus, dentate gyrus and parietal neocortex with that of control animals. A single dose of 3-NPA caused a significant increase of bcl-2 protein immunoreactivity in hippocampal neurons, i.e. CA 1 (5 out of 5 animals, p = 0.003), CA 3 (5/5, p = 0.003), CA 4 (4/5, p = 0.025), and neocortex (5/5, p = 0.004), in a time dependent manner over a period of 24 hr after injection. Neuronal bcl-2 protein expression in CA 2 and dentate gyrus remained unchanged. The data suggest a possible role of bcl-2-protein in chemical induction of ischemic tolerance using a single subtoxic dose of 3-NPA. Bcl-2-protein expression may be initiated by increased levels of reactive oxygen species (ROS) after 3-NPA administration, as shown by others. Additional bcl-2 protein may then be available to (1) control postischemic ROS burst, (2) protect the mitochondrial membranes, and (3) inhibit pro-apoptotic mechanisms.

But how does NPA inhibit? Alston and co-workers, based on some previous observations and on their own experience with another flavoprotein, proposed that the normal reaction pathway involves a temporary adduct with the N5 nitrogen of Flavin, which in the case of 3-NPA collapses to a stable adduct and permanent inactivation.

*Proc. Natl. Acad. Sci. USA*
Vol. 74, No. 9, pp. 3767–3771, September 1977
Biochemistry

# 3-Nitropropionate, the toxic substance of *Indigofera*, is a suicide inactivator of succinate dehydrogenase

**(rat liver mitochondria/carbanion/N-5 flavin adducts/two-proton abstraction mechanism)**

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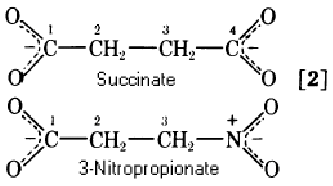
* Department of Biochemistry and Biophysics and † Harrison Department of Surgical Research, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104

*Communicated by Britton Chance, June 28, 1977*

**ABSTRACT** We have shown that 3-nitropropionate, an isoelectronic analogue of succinate, is a suicide inactivator of succinate dehydrogenase [succinate:(acceptor) oxidoreductase, EC 1.3.99.1] as follows. (*i*) When rat liver mitochondria oxidize succinate in the presence of 3-nitropropionate carbanion, the rate of $O_2$ consumption decreases exponentially to a zero value. This pattern is duplicated by subsequent additions of mitochondria. The dependence of the apparent first-order rate constant for enzyme inhibition, as well as the number of enzyme turnovers completed before inhibition, on the concentrations of 3-nitropropionate carbanion and succinate are those expected for an active site-directed and irreversible inhibitor. (*ii*) The inactivated enzyme is not resuscitated by centrifugation and washing of the mitochondria, in contrast to malonate-treated enzyme, and malonate protects against irreversible inhibition. (*iii*) The inhibitor species is 3-nitropropionate carbanion and no external nucleophile is required for inhibition. (*iv*) The respiratory rates, respiratory control ratios, and ADP/O ratios obtained with NAD-linked substrates are unaffected by 3-nitropropionate carbanion. These results show that 3-nitropropionate carbanion is a highly specific, time-dependent, and irreversible inhibitor of succinate dehydrogenase. By analogy with the reaction of nitroethane with D-amino acid oxidase, the data are consistent with the hypothesis that the carbanionic inhibitor forms a covalent N-5 adduct with the active site flavin. However, the precise mechanism of inactivation, as well as mechanistic extrapolations to the oxidation of succinate, must await the elucidation of the structure of the modified enzyme. We can now explain the toxicity of plants such as *Indigofera endecaphylla* for mammals and fowl as being due to the irreversible blockage of the Krebs cycle by 3-nitropropionate carbanion.




[2]

Singer and coworkers showed that the flavin was not irreversibly modified, and proposed that nitropropionate is oxidized to nitroacrylate, a reactive compound that modifies an essential residue in the active site. The suggested this was the essential sulfhydryl residue then believed to reside in the active sight and to be involved in the tight binding of OAA.

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 254, No. 12, Issue of June 25, pp. 5161-5167, 1979
*Printed in U.S.A.*

# Inactivation of Succinate Dehydrogenase by 3-Nitropropionate*

(Received for publication, November 20, 1978)

**Christopher J. Coles, Dale E. Edmondson, and Thomas P. Singer**

*From the Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143, and the Molecular Biology Division, Veterans Administration Hospital, San Francisco, California 94121*

This paper examines proposals in the literature that 3-nitropropionate is a suicide inactivator of succinate dehydrogenase and that it acts by nucleophilic addition to N-5 of the covalently bound flavin component of the enzyme. With purified, soluble preparations of the enzyme, the inhibition developed slowly, and nearly complete inactivation occurred with a stoichiometric amount of 3-nitropropionate dianion. These facts and the very slow oxidation of 3-nitropropionate by the enzyme (about 0.1% of the rate of succinate oxidation) seem to fit the usual criteria for an active site-directed suicide inhibitor. In accord with this, 3-nitroacrylate, the expected product of dehydrogenation by the enzyme, inactivates it extremely rapidly and irreversibly. Inactivation of the enzyme by 3-nitropropionate is accompanied by changes in the absorption spectrum of the enzyme and a loss of its slight flavin-type fluorescence. The spectral changes are not those expected from alkylation of N-5 of the flavin but are similar to the perturbations caused by binding of oxalacetate at the substrate site. Moreover, on denaturation of the enzyme or proteolytic digestion, even in anaerobic conditions, the absorption and fluorescence spectra of the oxidized flavin are observed. This is contrary to the behavior expected of an N-5 alkylated flavin. Several lines of evidence suggest that the oxidation product, 3-nitroacrylic acid, reacts with an essential —SH group at the substrate site. Thus, prior treatment with 3-nitropropionate prevents the binding of $^{14}$C-labeled oxalacetate at the substrate site, and, conversely, prior binding of oxalacetate to the enzyme prevented the irreversible inactivation by a 2-fold excess of 3-nitropropionate. Inactivation of the enzyme by 3-nitropropionate also prevented the alkylation of one —SH group by *N*-ethyl[$^{14}$C]maleimide. The —SH group in question is located in the 70,000-dalton subunit and is known from prior studies to be the combining site of succinate and of oxalacetate. It is suggested that the inactivation step involves a nucleophilic attack by this essential —SH group on the double bond of 3-nitroacrylate.

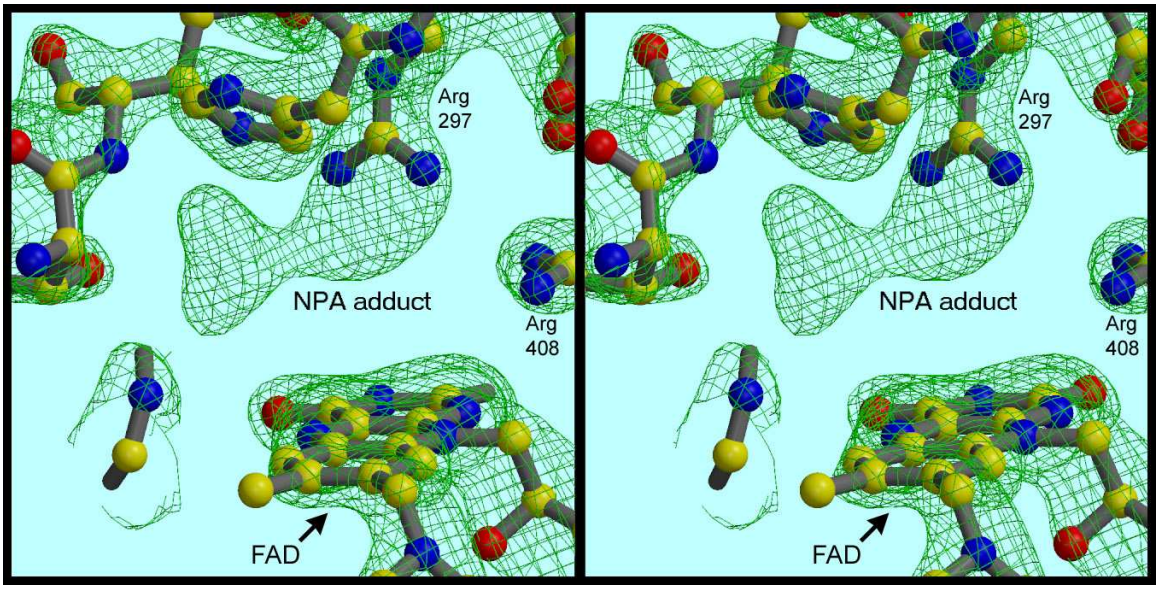



**Figure 24**. Density in the substrate binding site-

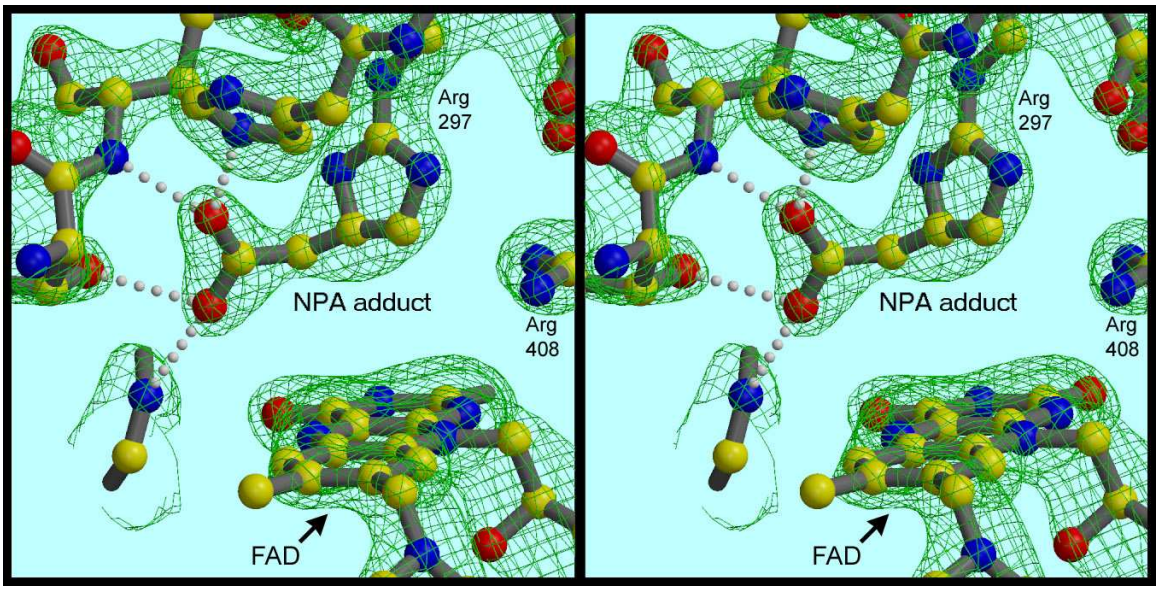



**Figure 25.**

modeled as a cyclic adduct of catalytic-base Arg297. We're not sure where the N of NPA goes- it could be the carboxylate is really nitrate or, if the inhibitor binds other way around, the ring could be 1,2,4 triazin instead of imidazole.

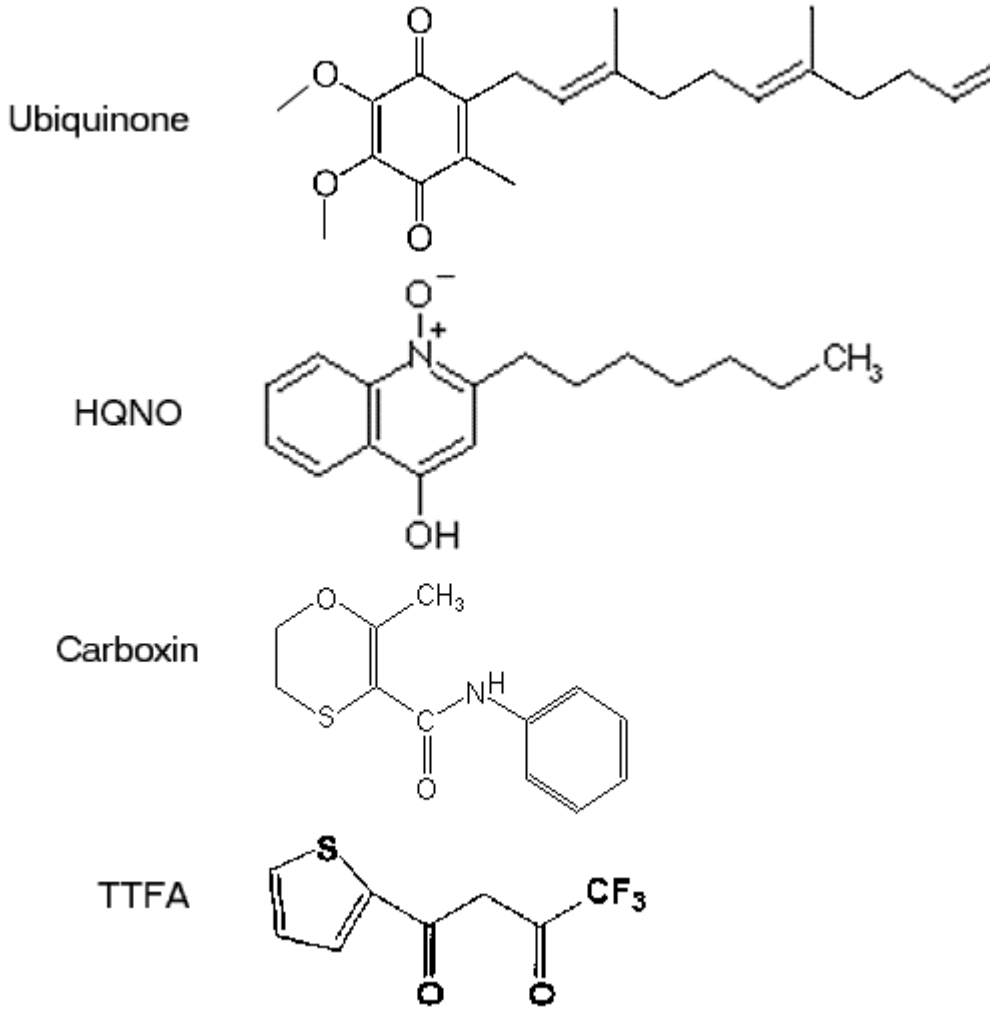



**Figure 28: Ubiquinone and Q-site inhibitors of Complex II.** Ubiquinone is substrate at the Q site. HQNO is a general Q analog, inhibiting at Q sites in a number of enzymes. It clearly resembles quinone, with O atoms para to each other across a 6-membered aromatic ring, a hydrophobic tail in the same place, and a second ring in place of the methoxy groups. Carboxin and TTFA are specific for the complex II Q site. 1NEK.pdb has a model for quinone binding at the Q site of Complex II which is unusual in that only one carbonyl O is involved in binding. If this is the case it seems likely that the carbonyl oxygens of carboxin and TTFA mimic that one keto group and the S-containing ring mimics the methoxy groups, with H-bond acceptors at the position of one or both methoxy oxygens.

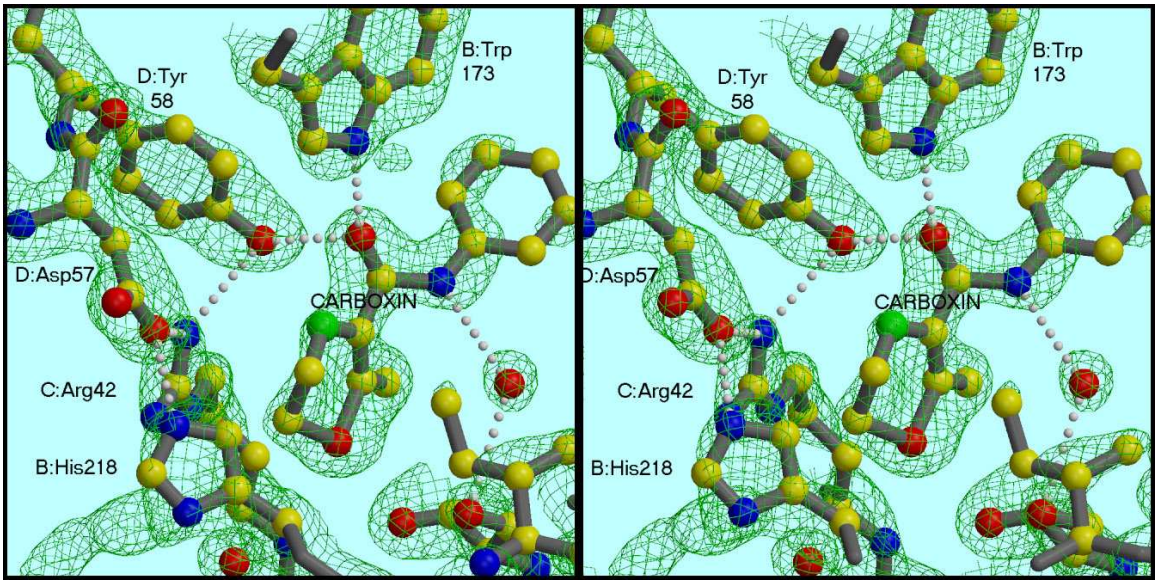


Figure 27. The electron density of the carboxin-inhibited enzyme in the region of 1NEK's Q-binding site supports the hypothesis that the carbonyl group mimics one carbonyl of quinone, H-bonding to Trp173 of chain B and Tyr58 of chain D.
Also visible are highly conserved C:Arg42, D:Ser39 (unlabeled), D:Asp57, B:His218