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Biochimica et Biophysica Acta 1364 (1998) 222–235

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Inhibitors of NADH–ubiquinone reductase: an overview

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Received 10 October 1997; revised 5 December 1997; accepted 9 December 1997

Abstract

This article provides an updated overview of the plethora of complex I inhibitors. The inhibitors are presented within the broad categories of natural and commercial compounds and their potency is related to that of rotenone, the classical inhibitor of complex I. Among commercial products, particular attention is dedicated to inhibitors of pharmacological or toxicological relevance. The compounds that inhibit the NADH–ubiquinone reductase activity of complex I are classified according to three fundamental types of action on the basis of available evidence and recent insights: type A are antagonists of the ubiquinone substrate, type B displace the ubisemiquinone intermediate, and type C are antagonists of the ubiquinol product. © 1998 Elsevier Science B.V.

Keywords: NADH–ubiquinone reductase; Respiratory inhibitor; Ubiquinone

1. Introduction

NADH–ubiquinone reductase (EC 1.5.6.3) is the energy-conserving enzyme complex that is commonly known as complex I [1–3]. Complex I embodies the first phosphorylation site of mitochondria and is first among the respiratory complexes also in the number of inhibitors. Over sixty different families of compounds are known to inhibit complex I, but recent articles have considered only a fraction of these [4–8], while the reviews on complex I inhibitors are becoming dated [9,10]. The purpose of this article is to provide an overview of the plethora of complex I inhibitors. To concisely present a large body of information, I introduce first a broad distinction among compounds of natural and commercial origin, and then propose a new functional classification of complex I inhibitors based on ubiquinone (Q) reaction sites.

2. The plethora of complex I inhibitors

The number of compounds that inhibit complex I is ever increasing, and it is impossible to classify them in chemical terms. Tables 1 and 2 report the

Abbreviations: CCCP, carbonyl cyanide-*m*-chlorophenylhydrazone; DB, decyl-Q analogue; DCCD, docyclohexylcarbodiimide; DCIP, dichlorophenolindophenol; DNP, 2,4-dinitrophenol; DPI, diphenyleneiodonium; DQ, duroquinone; I_{50}, inhibitor concentration producing 50% inhibition; HPP^+, 4-(4-chlorophenyl)-1-4-(4-fluorophenyl)-4-oxobutyl-pyridinium; MOA, methoxyacrylate; MPP^+, 1-methyl-4-phenyl-pyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,4,-tetrahydropyridine; 2M-TIO, 2-methyl-6-(2-thienyl)imidazo[2,1-*b*]thiazole; NP, 4-nonylphenol; Q, ubiquinone; Q-1, Q-2, ubiquinone-1, -2; QH_2, ubiquinol; RET, reverse electron transfer to NAD^+; SAN, Sandoz quinazoline compounds; SMP, submitochondrial particles; TDS, tridecyl stigmatellin; TIQ, tetrahydroisoquinoline; UBQ, undecyl-Q analogue

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PII S0005-2728(98)00029-2

diverse inhibitors divided into natural and commercial products with potency related to that of rotenone, the classical inhibitor of complex I [4,9,10]. The relative potency of natural inhibitors is often expressed in terms of the ratio with the K_I of rotenone (Table 1), that is 4 nM in our standard assay of NADH–Q reductase [8]. See Refs. [4,56] for a review on compounds that inhibit complex I among other enzymes, e.g. sulphydryl reagents.

3. Characteristics of complex I inhibition

A few compounds inhibit NADH interaction, but are not specific for complex I since they also affect other dehydrogenases (e.g. rhein [9,10,23]). Therefore, the great majority of complex I inhibitors act on the NADH–Q reductase activity, which is the limiting reaction in NADH oxidation [4,57,58], without inhibiting the NADH–ferricyanide reductase activity [4,9,59,13]. The inhibition of complex I can be measured in different ways. In coupled submitochondrial particles it is possible to follow the energy-dependent reverse electron transfer (RET) to NAD^+ [60,61]. RET assays are delicate and have been employed in toxicological studies [62]. Dichlorophenolindophenol (DCIP) and naphtoquinones such as menadione have been used as artificial electron acceptors for complex I activity [5,31,33,55,63,64], but their physiological significance is limited [4,56]. NADH oxidase or NADH–Q reductase are the recommended assays for a proper evaluation of complex I inhibitors. The NADH–Q reductase activity is best assayed with decyl-Q (DB) or undecyl-Q (UBQ) as electron acceptor substrates [25]. The I_{50} values of inhibitors observed with these Q analogues are generally larger than those obtained in the NADH oxidase assay [31] or with the hydrophilic Q-1. This latter Q analogue produces fast rates [25,48,63,65,66] but reacts incompletely with complex I [25,67].

Submitochondrial particles (SMP) are the system of choice in the study of complex I inhibition [4,8,56]. In fact, isolated complex I is largely inactivated by detergent extraction and its reactivity with quinones and inhibitors is substantially altered [4,56]. Beef heart SMP usually have 0.03–0.04 nmol per mg of protein of active complex I [4,8,13,59] (see, however, Ref. [68]). This implies that the minimal I_{50} for a potent inhibitor is around 0.02 nmol/mg and the observed I_{50} values approach the true K_I at particle concentrations below 0.02 mg/ml [8,68]. Because the minimal concentration of complex I that allows reliable measurements is 0.1 nM, the sub-nanomolar I_{50} values reported in [6,15] may reflect stoichiometric inhibition.

With regard to species-specificity, insect and fish mitochondria are particularly sensitive to complex I inhibition [6,33,69], which rationalizes the traditional use of rotenoids as fish and insect poisons [10,69]. By contrast, mitochondria of fungi and plants are naturally resistant to complex I inhibitors [7,22,70]. Among bacteria, *R. capsulatus* and *P. denitrificans* display inhibitory sensitivities that are close to those of mammalian mitochondria [7,54,71,72], but *E.coli* is extremely insensitive to rotenone and other inhibitors [7,73]. At the cellular level, complex I function is particularly crucial in tissues such as neurons and pancreatic islet beta cells that heavily rely upon mitochondrial ATP and NAD-linked pathways [74–76]. The potent cytotoxic effect of inhibitors in cancer cell cultures [77–79] suggests that complex I activity is delicate also in transformed cells, probably because they usually have a smaller number of mitochondria than their parent normal cells.

4. Complex I inhibitors of natural origin

In essence, the structure of potent natural inhibitors of complex I has a modular similarity with ubiquinone, with a cyclic ‘head’ corresponding to the ubiquinone ring and a hydrophobic ‘tail’. Natural variations within each family of inhibitors is normally restricted to substitutions and degrees of oxygenation of the tail (see also the contribution of Miyoshi [80]).

4.1. Rotenoids

Rotenone is the most potent member of the rotenoids, a family of isoflavonoids extracted from *Leguminosae* plants [12,70,81], and since the early reports [69,81] has become the classical inhibitor of complex I [1,4,10,56,68]. Rotenone inhibition is markedly time-dependent [4,9,12], which easily leads to under-estimations of its potency—a K_I as low as

Table 1
Complex I inhibitors of natural origin and their analogues

Family of compounds	Source	Potent representative	I_{50} values in beef mitochondria	K_I (M)	Assay system	Relative potency [a]	Notes and Refs.
Rotenoids	plants (e.g. *Derris*)	Rotenone	0.07 nmol/mg 0.04 nmol/mg	4 nM	NADH–Q NADH–O_2	1	insecticide and pesticide [7,8,11]
		Deguelin	4.2 nmol/mg		NADH–Q	0.02	insecticide [12]
	synthetic	Analogue 13	0.35 nmol/mg		NADH–Q	1	
Piericidins	*Streptomyces*	Piericidin A	0.06 nmol/mg 0.03 nmol/mg	1.0 nM 0.6 nM	NADH–Q NADH:O_2	> 4	two sites, Q antagonist[b] [7,8,13]
	synthetic	Ubicidin-3	0.17 nmol/mg	1.5 nM	NADH:O_2	2	[14]
	synthetic	hydroxypyridine analogue 21	0.23 nmol/mg		NADH:O_2	1	[15]
Annonaceous acetogenins	plants (e.g. *Annona*)	Rolliniastatin-1	0.03 nmol/mg	0.3 nM	NADH–Q	13	most potent and specific [8]
		Rolliniastatin-2 or Bullatacin	0.06 nmol/mg	0.6 nM	NADH–Q-1	6.7	action different from rotenone [8]
		Otivarin	0.2 nmol/mg	0.9 nM	NADH–Q	> 1	action similar to rotenone [8]
Myxobacterial antibiotics	*Polyangium*	Phenoxan	0.04 nmol/mg		NADH–Q	2	[7]
	Polyangium	Thiangazole	0.04 nmol/mg		NADH–Q	2	[7]
	Cystobacter	Myxalamid PI	15 nmol/mg		NADH–Q	0.006	[7]
	Myxococcus	Phenalamid A_2	0.6 nmol/mg		NADH–Q	0.12	[7]
	Stigmatella	Aurachin A	8.0 nmol/mg		NADH–Q	0.01	Q antagonist[b] [7]
	Myxococcus	Myxothiazol	20 nmol/mg	0.1 μM	NADH–Q	0.04	Q antagonist[b] [16,17]
	Stigmatella	Stigmatellin	100 nmol/mg		NADH–Q	0.004	Q antagonist[b] [16]
	synthetic	TDS	40 nmol/mg	0.2 μM	NADH–Q	0.02	Q antagonist[b] [16,17]
Other antibiotics	*Streptomyces*	Aureothin	0.3 nmol/mg		NADH–Q	0.23	[7]
	Bipolaris	Cochlioquinone B	83 nmol/mg		NADH:O_2	0.002	Q antagonist[b] [18]
	Strobilurus	Mucidin	> 100 nmol/mg		NADH–Q	0.001	Q antagonist[b] [16]
	Pterula	Pterulone	36 μM		NADH:O_2	< 0.002	antifungal [19]
Vanilloids	plants (e.g. *Capsicum*)	Capsaicin	20–30 μM		NADH–Q NADH:O_2	< 0.001	irritant, analgesic [20,21]
	synthetic	analogue 37	6.2 nmol/mg		NADH–Q	0.27	[22]
Plant products	rhubarb	Rhein	30 μM	2 μM	NADH:O_2	< 0.002	NADH antagonist[c] [23]
	opium	Papaverine	5 μM		NADH–Q-1	0.006	vasodilator [24]
Ubiquinones	synthetic	Ubiquinone-2	2 μM		NADH–Q-1	0.003	[25,26] [27,28]
	synthetic	Ubiquinone-3	40 μM		NADH–Q	< 0.001	[26,27]
	synthetic	Idebenone	0.4 μM		NADH–Q	0.01	nootropic drug [28]

1 nM has been reported [68]. Detailed measurements with [^{14}C]rotenone have indicated a specific binding titre of 0.06–0.07 nmol/mg protein in beef heart SMP [82] and I_{50} values approach this titre when the particle concentration is above 0.1 mg/ml in the assay. Binding studies by Singer and coworkers have indicated that amytal, piericidin A and 1-methyl-4-phenyl-pyridinium (MPP^+) displace rotenone from its specific site in complex I [4,9,56,82]. Results with photoaffinity labelling [83], fluorescent dyes [84] and [^{3}H]dihydrorotenone [6,85] have later clarified that rotenone does not compete with Q analogues. Photoaffinity labelling data have suggested that the ND1 subunit could be directly involved in forming the rotenone binding site [86,87]. Investigations on human disease mutations partly sustain this possibility and indicate that also the ND4 subunit could be involved in rotenone binding [88,89].

4.2. Piericidins

Piericidins are 2,3-dimethoxy-4-hydroxy-5-methy-6-polyprenyl-pyridine antibiotics produced by some *Streptomyces* strains [90] that have contributed extensively to the definition of the enzyme properties of complex I [4,9–11,13,14,56,91–94]. The methylation of the hydroxyl group at position 10 of the tail in natural piericidin B and D seems to reduce potency [11,93], consistent with the diminished potency of the 6-isoprenyl derivatives of piericidins, usually called ubicidins [14,92]. The close similarity with ubiquinone renders piericidin A an effective inhibitor of several Q-reacting enzymes (see Ref. [16] and reference therein). Complex I, however, remains the most specific site of piericidin inhibition, with a K_I ranging between 0.6 [14,91] and 1 nM [8]. Studies with [^{14}C]piericidin A have indicated that complex I has two binding sites for the inhibitor in the native membrane [13,94], one of which inhibits energy-dependent RET more potently than NADH oxidase [94]. The tight binding of piericidin A to complex I essentially prevents its displacement by rotenone and amytal [4,9,11]. However, a partial displacement of bound piericidin A has been obtained with certain analogues of MPP^+ [4,95], whilst the acetogenin rolliniastatin-2 is mutually exclusive with piericidin A [8]. New information on the molecular interaction of piericidin will be hopefully obtained with the resistant mutants recently described in *Rhodobacter* [71].

4.3. Annonaceous acetogenins

Natural products called annonaceous acetogenins derive from *Annonaceae* plants such as custard apple [5,8,77,79,96,97]. They have been initially characterized as antitumoral agents [77,79], and subsequently recognized as specific inhibitors of mammalian complex I [5,8,97]. The family of annonaceous acetogenins now includes nearly 200 different compounds, some of which are extremely potent inhibitors [5,8,77,96–98]. Their global structure consists of an aliphatic backbone of 35–37 carbons terminating with a substituted γ-lactone and containing a central nucleus of one or two tetrahydrofuran groups [79,96]. Various hydroxyl groups are distributed along the molecule and seem to be essential for inhibition [5,77,96]. However, little information is available on the structure–activity of these inhibitors, in part due to the limited natural variations that are confined to the number of hydroxyl groups and the structure or stereochemistry of the tetrahydrofuran nucleus [77,79,96]. This tetrahydrofuran nucleus is likely to be fundamental for biological activity, since it could mimic the quinoid head of ubiquinone (cf. Ref. [5]). The part of the molecule with the lactone could correspond to the hydrophobic tail of other inhibitors such as piericidins, whereas the aliphatic part could essentially act as a lipophilic anchor.

Notes to Table 1:

[a] Potency relative to that of rotenone measured under the same conditions in beef mitochondria or submitochondrial particles. The relative potency is measured using the ratio of the K_I values, when they are available. NADH–Q reductase is with hydrophobic Q analogues such as DB, UBQ and Q-2.

[b] These inhibitors are general Q antagonists since they inhibit also other Q-reacting enzymes, especially cytochrome bc_1 complex and bacterial reaction centres.

[c] Rhein specifically inhibits the rotenone-insensitive reduction of ferricyanide and acts as a NADH antagonist in soluble mitochondrial NADH dehydrogenase, as well as in other dehydrogenases [9,23].

Table 2
Commercial and synthetic inhibitors of complex I

Type of compounds and main use	Representative inhibitor	Minimal I_{50}^{a}	Assay system	Relative potency[b]	Refs.
(a) Pesticides					
Acaricides and insecticides	Sandoz 547A	2 nM	NADH–Q-1	>2	this work
	Pyridaben	2.4 nM	NADH–Q-1	2	this work
	Fenpyroximate	4.6 nM	NADH–Q-1	1.1	this work
		18 nM	NADH–Q	1.4	[22]
	Tebufenpyrad	6 nM	NADH–Q-1	ca. 1	this work
	Fenazaquin	20 nM	NADH–Q-1	0.5	[5]
	Benzimidazole	3 nM	NADH–Q	1.4	[7]
Pyrethroid insecticides	Cyhalothrin	0.6 μM	NADH–DQ	4×10^{-2}	[29]
Insecticide synergists	6-chloro-benzothiadiazole	0.1 mM	NADH:O_2	1×10^{-4}	[30]
Antihelmintic mimics	2M-TIO (compound 10)	40 μM	NADH–Q-1	6×10^{-3}	[31]
(b) Drugs and toxins					
Sedative drugs	Amytal (barbiturate)	0.2 mM	NADH–Q-1	5×10^{-5}	[31,32]
Analgesic drugs	Meperidine (Demerol)	0.1 mM	NADH–Q-1	1×10^{-4}	this work
	Meperidine analogue 49	0.8 μM	NADH–Q	0.6	[33]
Synthetic neurotoxins	MPP^+	0.3 mM	NADH:O_2	3×10^{-5}	[34]
		2–4 mM	NADH–Q	$<10^{-5}$	[35,36] [37]
	decyl-MPP^+	1.7 μM	NADH:O_2	2×10^{-2}	[36]
	MQ18	0.2 μM	NADH–Q-1	0.1	[37]
	2-methylharmine	0.18 mM	NADH:O_2	6×10^{-5}	[38]
Natural neurotoxins	TIQ	2 mM	NADH:O_2	$<10^{-5}$	[39,40]
	Aminoethyl cysteineketamine	1.3 mM	NADH:O_2	$<10^{-5}$	[39]
Neuroleptic drugs	Haloperidol	3 μM	NADH–Q-1	2×10^{-2}	[41,42]
Drug metabolite	HPP^+	15 μM	NADH–Q-1	2×10^{-3}	[42]
Mouth wash antiseptic	Dequalinium chloride	11 μM	NADH:O_2	3×10^{-3}	[43]
Antihistaminic drugs	Cinnarizine	5–10 μM	NADH–Q-1	1×10^{-3}	[44]
Antianginal drugs	Ranolazine	23 μM	NADH–Q-1	2×10^{-3}	[45]
(c) Phenolic chemicals					
Rodenticides	Vacor	50 μM	NADH–Q	5×10^{-3}	[46]
Phenols: pollutant	nonyl-phenol	20 μM	NADH–Q	3×10^{-3}	this work
pollutant	catechol	0.4 mM	NADH–Q	2×10^{-5}	this work
uncoupler	dinitrophenol	0.15 mM	NADH–Q-1	1×10^{-4}	[47]
uncoupler	CCCP	85 μM	NADH–Q-1	2×10^{-4}	this work
(d) Q antagonists					
Acridones	4-*s*-butyl analogue	1.4 μM	NADH–Q-1	5×10^{-2}	[48]
	7-chloro-4-octyloxy analogue	0.2 μM	NADH–Q-1	<0.1	[48]
Quinolones	2-undecyl-3-methyl analogue	20 nM	NADH–Q-1	ca. 1	[49]
Quinolines	4-hydroxy analogue 37	10 nM	NADH:O_2	ca. 1	[15]
Phenylpyridines	4′-heptyl analogue	1.7 μM	NADH:O_2	2×10^{-2}	[36]
(e) Fluorescent dyes					
Fluoresceins	Erythrosin 5′-iodoacetamide	20 nM	NADH–O_2	0.8	[50]
Acridines	Safranine	17 μM	NADH–Q	2×10^{-3}	[51]
Carbocyanines	$diOC_5(3)$ (D-272)	1.5 μM	NADH–Q	5×10^{-2}	[51]
	$diOC_6(3)$	80 nM	NADH–O_2	0.2	[50]
(f) Group reagents					
Flavin reagent	DPI	0.23 μM	NADH–Q-1[c]	<0.1	[52]
Carboxyl reagent	DCCD	0.1 mM	NADH–Q-1	1×10^{-4}	[53]
Metal chelator	*o*-phenantroline	0.25 mM	NADH–Q	4×10^{-5}	[54]

Acetogenins with two adjacent tetrahydrofurans such as rolliniastatin-1 and rollimembrin are the most potent inhibitors of complex I, with K_I values around 0.3 nM [8,98]. Next in potency is rolliniastatin-2 or bullatacin [5,8,77,96], which has a K_I similar to that of piericidin A (Table 1) and is consistently non-exclusive with rotenone [8]. Indeed, the sensitivity to rolliniastatin-2 is unaltered in *Rhodobacter* mutants [71] and human pathological conditions showing resistance to rotenone [99]. Besides rolliniastatin-2 [8], a few acetogenins are likely to be non-exclusive with rotenone (E. Estornell, personal communication), while many others seem to act like rotenone, depending in particular on the presence of an hydroxy group near the substituted lactone (e.g. squamocin or otivarin vs. rolliniastatin-2 [8]).

4.4. Myxobacterial antibiotics

Myxobacteria are a precious source of potent mitochondrial inhibitors [100]. Myxothiazol and stigmatellin are most specific for cytochrome bc_1 complexes, but inhibit also complex I [16]. Myxothiazol has a K_I of 0.1 μM in beef SMP [16,17] and is more potent than stigmatellin (Table 1). Both inhibitors seem to compete for a common site in complex I which overlaps that of rotenone only in part [6,17] and specifically interferes with the ubiquinol product [31,58]. Myxobacteria produce several other antibiotics that inhibit complex I: aurachins [100], myxalamid [101], phenalamid [102], phenoxan [103] and thiangazole [104]. The quinolone aurachins have been found to be more specific for bacterial quinol oxidases and cytochrome *bc* and *bf* complexes [105] than for complex I (cf. Ref. [7]). Conversely, phenoxan and thiangazole seem to be most specific for complex I, with a potency about two-fold stronger than that of rotenone in mammalian mitochondria [7] (Table 1).

4.5. Other antibiotics

Table 1 reports other antibiotic inhibitors such as aureothin {(*Z,E*)-2-methoxy-3,5-dimethyl-6-[tetrahydro-4-[2-methyl-3-(4-nitrophenyl)-2-propenylidene]-2-furanyl]-4h-pyran-4-one} [106] which seems to act like rotenone [7]. Among cochlioquinones, a family of fungal toxins of polycyclic structure, cochlioquinone B seems to be specific for the NADH–Q reductase activity [18].

4.6. Vanilloids and other plant products

Capsaicin {(*E*)-*n*-[(4-hydroxy-3-methoxyphenyl)-methyl]-8-methyl-6-nonenamide} is the most representative member of the vanilloids, pungent substances extracted from hot peppers [107,108]. Vanilloids are widely used in neurobiology studies (see Ref. [107] for a review), and have applications as topical analgesics, food additives and even sprays for personal defence. The mitochondrial toxicity of capsaicin derives from its inhibition of complex I [20]. Compared to other natural products, capsaicin is a weak inhibitor (I_{50} values around 30 μM in mammalian SMP [7,21,22,31]), but shows the rare property of competitive inhibition vs. Q substrates in isolated complex I [20]. In SMP, the kinetic behaviour of capsaicin is biphasic or mixed-competitive as for several other inhibitors (cf. Refs. [21,22]). Table 1 lists other plant products that do not belong to families of specific inhibitors of complex I such as papaverine, a compound present in opium [24].

4.7. Ubiquinones

Short-chain quinones such as Q-2, Q-3 and octyl-Q function both as substrates and inhibitors of NADH oxidation in mammalian mitochondria [25–28,67]. These quinone analogues accept electrons from complex I and become inhibitory once reduced [25,27], a

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Notes to Table 2:

[a] I_{50} minimal in beef or rat mitochondrial preparations with the assay system indicated (NADH:O_2 represents both NADH oxidase determined spectrophotometrically and site one respiration determined with the oxygen electrode). Due to the abundance of data, the major reference assay is NADH–Q-1 reductase.

[b] Relative to the I_{50} of rotenone assayed under the same conditions.

[c] DPI inhibits also the oxidation of NADH by either ferricyanide or DCIP in the absence of exogenous Q analogues, especially in purified complex I [55].

suicide-like action that seems to derive from semiquinone instability, since it increases oxygen radical production in complex I [25,28]. The hydroxydecyl Q analogue idebenone is more potent than Q-2 and specifically inhibits membrane potential [28], which raises some worries for its use as a nootropic drug [109].

5. Synthetic and commercial inhibitors

5.1. Pesticides and agrochemicals

A new series of pesticides inhibiting complex I has been developed in the last few years [5,6] and now commercialized as acaricides. SAN547A and SAN548A [5] are *N*-alkyl-4-aminoquinazolines related to the pesticide fenazaquin [110–112] and probably constitute the most potent synthetic inhibitors of complex I (Table 2). Fenpyroximate {*tert*-butyl (*E*)-α-(1,3-dimethyl-5-phenoxypyrazol-4-ylmethyleneamino-oxy)-*p*-toluate} [113] is the only new pesticide mentioned in the latest Merck Index (1996) and inhibits beef complex I with a potency equivalent to that of rotenone (Table 2, cf. Refs. [7,22,113]). Although fenpyroximate displaces labelled dihydrorotenone [6], it has been suggested to act differently from rotenone, mainly because it inhibits bacterial glucose dehydrogenase like piericidin [7]. Pyridaben {2-*tert*-butyl-5-(4-*tert*-butylbenzylthio)-4-chloropyridazin-3(2*h*)-one} is a pyrazole acaricide that seems to be superior to rotenone as inhibitor of mammalian complex I [5,114] (Table 2). Pyridaben is also more potent than tebufenpyrad [115] {*n*-(4-*tert*-butylbenzyl)-4-chloro-3-ethyl-1-methyl-5-pyrazolecarboxamide} (Table 2) that is commercialized with the tradename pyranica [116,117].

Among benzimidazole compounds with insecticidal properties, 1-(3,7-dimethyl-7-isobutyloxy-2-octenyl)-2-methylbenzimidazole [118] seems to be more potent than rotenone in mammalian mitochondria (Table 2, cf. Refs. [7,118–120]). Related to benzimidazoles are the insecticide synergistic 6-chloro-1,2,3-benzothiadiazole [30] and various substituted imidazo-thiazoles designed to mimic anthelmintic drugs [31,121]. 2-methyl-6-(2-thienyl)imidazo[2,1-*b*]thiazole (2M-TIO) seems to functionally compete with myxothiazol and rotenoids, but not rolliniastatin-2 [31]. Recently, pyrethroid insecticides such as cyalothrin have also been found to inhibit complex I [29].

5.2. Drugs and toxins

Whereas complex I is the target for new pesticides in agrochemistry, complex I inhibition is an increasing toxicological problem, since it constitutes an undesired side-effect of several drugs used in medicine. Indeed, many pharmacologically important compounds inhibit NADH–Q reductase and their potency in isolated mitochondria, albeit weak when compared with acaricide inhibitors (Table 2), is not directly proportional to the pathological effects [34–37,122].

5.2.1. Tranquillizers

The tranquillizer barbiturate amytal (5-ethyl-5-isoamylbarbituric acid) is the oldest established inhibitor of complex I [4,9,10,32]. Meperidine {1-methyl-4-phenyl-4-piperidinecarboxylic acid ethyl ester}, also called pethidine or demerol, is another analgesic drug known to inhibit complex I [33,63]. Werner and coworkers have synthetized hydrophobic analogues which are about 100-fold more potent than pethidine in inhibiting NADH–Q reductase [33] and a radioactive analogue which apparently is not displaced by rotenone [123]. This agrees with enzymatic data showing that meperidine or demerol is non-exclusive with rotenoids and other inhibitors of complex I [17].

5.2.2. Neurotoxins

The neurotoxin 1-methyl-4-phenyl-1,2,3,4,-tetrahydropyridine (MPTP) induces parkinsonism in humans once transformed into the pyridinium metabolite MPP^+, which is responsible for the specific toxicity in dopaminergic neurons [34,35,122]. MPP^+ is a rather weak inhibitor in broken mitochondria or SMP, but in coupled mitochondria is actively accumulated in the matrix due to its positive charge, with a dramatic increase in potency [34–36,122]. Some 4′-alkyl analogues of MPP^+ are inhibitory in the micromolar range [36,37] and displace either rotenone or piericidin A from its binding site in complex I [4,95,124,125], but seem to have two distinct interaction sites [36,37,95]. Extensive research has been undertaken to find compounds with an action similar

to that of MPP^+ and hopefully rationalize the spontaneous occurrence of Parkinson's disease. Potential candidates include indole-derived-β-carboline compounds such as harmane [38], the antiseptic dequalinium [43], laser dyes [35] and the naturally-occurring aminoethylcysteine ketimine dimer [39] and tetrahydro-isoquinoline (TIQ) [24,126,40]. Finally, dopamine and other catecholamine neurotransmitters have well known neurotoxic effects [127] that are possibly correlated to complex I inhibition [24,127,128]. This correlation, however, is not proven yet.

5.2.3. Neuroleptic drugs

Anti-psychotic or neuroleptic drugs have long been suspected to interfere with mitochondrial function [129,130]. The common side-effects of neuroleptic treatment include parkinsonism [131], which has been recently correlated to complex I inhibition in isolated mitochondria [41]. Further correlations between the side-effects of neuroleptic medication and parkinsonism derive from the discovery of pyridinium metabolites of the drug haloperidol {4′-fluoro-4-(4-hydroxy-4-*p*-chorophenylpiperidino) butyrophenone} in patients [132,133]. These metabolites resemble MPP^+ not only in structure, but also in their mode of complex I inhibition [42].

5.2.4. Piperazine drugs

Recently, some commercial drugs derived from piperazine (diethylenediamine) have been found to inhibit complex I in the micromolar range (Table 2, cf. Refs. [44,45]). They include the anticonvulsant/antihistaminic cinnarizine [44] and the antianginal ranolazine {1-[3-(2-methoxyphenoxy)-2-hydroxypropyl]-4-[*N*-(2,6-dimethylphenyl)carbamoylmethyl]piperazine} [45]. Ranolazine has some unusual features as complex I inhibitor, since it displays an uncompetitive behaviour with respect to Q-1 [45] similarly to annonaceous acetogenins [8].

5.3. Phenolic chemicals

Besides catechol, several other phenolic compounds inhibit complex I. 4-nonylphenol (NP), a pollutant derived from non-ionic detergents, is more potent than 2,4-dinitrophenol (DNP) and other uncouplers such as carbonyl cyanide-*m*-chlorophenylhydrazone (CCCP) (Table 2). NP appears to compete with quinol product inhibitors in complex I and is mutually exclusive with stigmatellins and myxothiazol (A. Ghelli, M. Degli Esposti, unpublished data). A series of 2-alkyl-dinitrophenols has been developed as general Q antagonist inhibitors, but their inhibition of complex I is only partial [47]. Related to nitrophenols is Vacor {1-(4-nitrophenyl)-3-(3-pyridylmethyl)urea}, a rat poison that induces an acute condition of insulin-dependent diabetes in humans [134]. Complex I inhibition is responsible for the diabetogenic action of Vacor [46].

5.4. Quinone antagonists

Oettmeier and coworkers have synthesized new quinone-related inhibitors [48,49,135]. Acridones with a 4-alkyl tail of eight carbons display maximal potency for complex I, which is about one-order of magnitude lower than that of rotenone [48,135]. Binding studies have shown an apparent displacement of acridones by rotenone [6,135]. The 2-*n*-undecyl-3-methyl analogue is the most potent of the quinolone series, even more than its related aurachins in both complex I and cytochrome bc_1 complexes [49]. The acridone and quinolone inhibitors presumably act as Q antagonists in complex I, but they do not behave competitively with Q-1 [48].

5.5. Fluorescent dyes

Acridones are closely related to acridines and fluoresceins, which include many fluorescent dyes used in biological studies. The mitochondrial toxicity of potential-sensitive dyes such as carbocyanines has long been known [136] and essentially derives from complex I inhibition, as established in extensive studies [50,137–139]. Erythrosine iodoacetamide, a fluorescein derivative, probably is the most potent dye inhibiting complex I (Table 2, cf. Refs. [50,84]); its binding to isolated complex I is mutually exclusive with DB but not with rotenone [84]. It is noteworthy that among oxonol dyes, including tinopal [72], only oxonol-VI does not inhibit complex I.

5.6. Group reagents

The classical carboxyl reagent dicyclohexyl-carbodiimide (DCCD) [53] and the metal chelator *o*-

phenanthroline have been found to inhibit complex I, as well as other membrane systems [54,140]. Conversely, the flavin reagent diphenyleneiodonium (DPI) inhibits complex I with high potency [55]. Although originally reported as inhibitor of the NADH–Q reductase activity [52], DPI covalently attacks the FMN cofactor after reduction of isolated complex I and thus blocks electron transport to hydrophilic acceptors such as DCIP [55].

6. Functional classification of inhibitors of NADH–Q reductase

In examining the variety of complex I inhibitors it emerges that several compounds seem to act differently from rotenone. Rotenone itself is likely to bind to two sites [4,68,94], as originally indicated by binding studies with piericidins [11,13,14,91]. Indeed, concentrations of rotenone that saturate its specific site do not significantly displace bound piericidin A [11,13], which suggests that the binding of the two inhibitors is either independent or overlapping only in part. Moreover, some new inhibitors such as myxothiazol are non exclusive with either rotenone or piericidin [17,58]. How can we distinguish functionally different sites for complex I inhibitors?

There is an increasing consensus of opinion that complex I has multiple Q reaction sites, but it is arduous to define a clearcut distinction among these sites. Perhaps most compelling is the theoretical consideration that no less than two Q action sites need to be present in complex I to account for the generally accepted stoichiometry of two protons per electron [1–4,58]. A consequence of this minimal requirement is that some inhibitors could interfere with protonmotive reactions differently from other inhibitors depending on their interaction with a given Q site. In fact, one of the two piericidin A sites affects more potently energy-dependent RET than NADH oxida-

Table 3
Functional classification of complex I inhibitors

Inhibitor type and action	Representative inhibitors	Likely inhibitors
Type A—quinone antagonists	Rolliniastatin-2	other annonaceous acetogenins
	Piericidin A (first site)[a]	Erythrosin iodoacetamide
	Idebenone	Aurachin A
		Phenalamid
		Ranolazine
Type B—semiquinone antagonists	Rotenone	other rotenoids
	Piericidin A (second site)[a]	Ubicidins
	Piericidin B	Reduced Q-2[b]
	Aureothin	4-alkyl-quinazolines (SAN 547A)
	Amytal	Pyrazole acaricides
	4-alkyl-acridones	Squamocin
	4′-alkyl-MPP$^+$ analogues[c]	Otivarin
	Phenoxan	Quinolones
Type C—quinol antagonists	Quinol products	4′-alkyl-MPP$^+$ analogues[c]
	reduced Q-2[b]	4′-alkyl-pyridines
	Myxothiazol	Myxalamid
	Stigmatellin	Vacor
	TDS	Carbocyanine dyes
	2M-TIO	Acridine dyes
	Meperidine (Demerol)	DNP and other uncouplers
	NP	

[a] Piericidin A has two sites [4,13,14,94].
[b] Q-2 forms unstable semiquinones upon reduction and leads to quinol occupation of the site where normally hydrophobic Q analogues enter complex I (Fig. 1, cf. Ref. [28]). Reduced Q-2 also acts at the quinol site.
[c] 4-alkyl analogues of MPP$^+$ have two sites of interaction [36,37].

tion [13,94]; most likely, rolliniastatin-2 specifically interacts at this site [8,58]. By contrast, rotenone inhibits more effectively NADH oxidation than energy-dependent RET [8,68,141] and is less efficient than rolliniastatin-2 in inhibiting the proton pumping activity of complex I under limited turnover conditions [58]. Rotenone and piericidin A also differ in their kinetic behaviour towards Q analogue substrates. Rotenone is consistently non-competitive [6–8,31,33,22,48,45], whereas piericidin A is competitive with endogenous Q [91,14,94] and partially competitive with some exogenous Q analogues [7]. Note

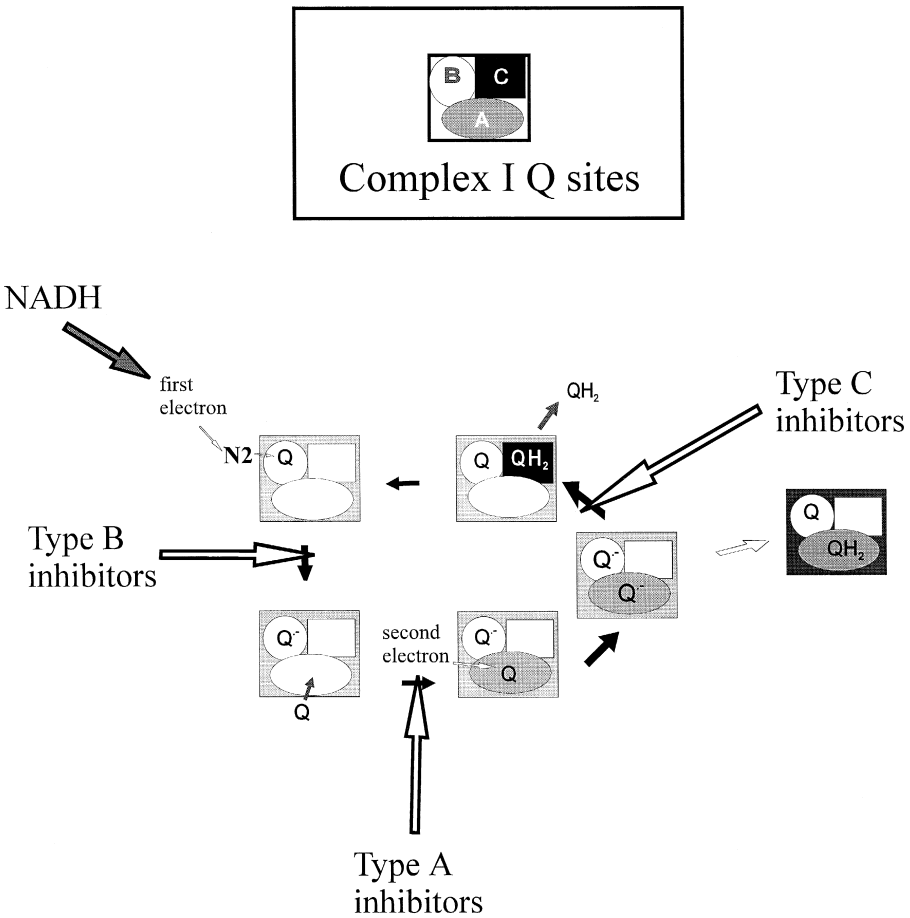

Fig. 1. A diagram for the action of the three fundamental types of inhibitors of NADH–Q reductase. The diagram represents the minimal steps of Q reduction by complex I and derives from the dual Q-gated pump model [58] modified to account for the likely protonmotive role of cluster N2 [3]. The first electron from NADH, through oxidoreduction of cluster N2, reduces a firmly bound Q molecule at site B, forming a stabilized ubisemiquinone that corresponds to the rotenone-sensitive radical [142–144], and produces some charge separation [51]. The second electron from NADH is transferred by the low-potential cofactors to another molecule of ubiquinone which enters the complex at the hydrophobic site A and activates proton pumping [58]. Rolliniastatin-2 and other type A inhibitors antagonize Q at this site. The second semiquinone formed at site A then dismute with the more stable semiquinone at site B, producing the ubiquinol product that leaves the complex. Oxidized Q remains at site B after dismutation to re-initiate the catalytic cycle. The dismutation of the two semiquinones is a critical step in the proper function of complex I [143]. With exogenous analogues such as Q-2 that have a pronounced semiquinone instability, the dismutation step can diverge from the normal route, producing quinol at the 'wrong' site, as illustrated by the white arrow and the rightmost enzyme form in the diagram. I hypothesize that type C inhibitors act by effectively enhancing this unproductive form of the enzyme at steady-state. To do so, they could either interact directly with the quinol-releasing site, or interfere with the dismutation reaction of the semiquinones in sites A and B. Presumably, the dismutation reaction of the two semiquinones is also associated with charge separation across the membrane, and its destabilization may enhance the production of oxygen radical species by complex I.

that rolliniastatin-2 shows competitive behaviour with idebenone and decyl-plastoquinone (M. Degli Esposti, A. Ghelli, unpublished data) but uncompetitive behaviour with other Q analogues [8].

By combining these different properties, complex I inhibitors can be broadly classified into inhibitors acting like rolliniastatin-2 or piericidin A (at one of its two sites) and inhibitors acting like rotenone (at its most specific site). These two types of inhibitors would partially correspond to class I and II, respectively, that were proposed previously [7] and include several other compounds chemically unrelated to rotenoids or piericidins. However, there are some inhibitors that fit neither the rotenone nor the rolliniastatin-2/piericidin A type. For instance quinol analogues, especially $Q2H_2$, are particularly potent for the membrane potential generation of complex I and the redox activity with hydrophilic substrates such as Q-1, that promote inefficient proton pumping [25,67] —presumably because these quinones are protonated in the aqueous phase and not within the membrane like natural ubiquinone. Myxothiazol, stigmatellin, NP, capsaicin, demerol and some cationic MPP^+ analogues also appear to interact with a hydrophilic site in complex I [17,37] and share the inhibitory properties of quinols. Hence, these compounds could form a third type of complex I inhibitors which essentially act as the quinol product [58].

Given the above, I propose a functional classification of various complex I inhibitors into three fundamental types (Table 3) that interfere with basic steps in Q reduction as schematically illustrated in Fig. 1. In brief, they act as: A, direct antagonists of Q substrate at the entry of the hydrophobic site in the complex; B, antagonists of the semiquinone intermediate stabilized within the complex; and C, antagonists of either the formation or release of the quinol product. Different chemical determinants are associated with these three types of inhibitors. Type A inhibitors possess a hydroxyl group both in the cyclic head and the hydrophobic tail, optimally spaced by 10–12 carbons as in piericidin A, rolliniastatin-2 and idebenone. Conversely, type B inhibitors have only H-bonding acceptors in the head and no hydroxy groups in the tail. In fact, even piericidins without the hydroxyl group in the tail displace bound rotenone but not bound piericidin A [11,14]. By using these inferences and earlier data [7], phenalamid, aurachins and also erythrosin could be classified as type A inhibitors. Type C inhibitors, instead, generally possess a hydroxy or amino group in the head. However, the hydrophilic site at which they are supposed to interact is likely to have a rather weak specificity for recognizing chemically different compounds, and may as well accept substances that bind with more affinity to other sites in complex I, e.g. rotenone.

Two qualifications are required with regard to the new classification proposed here (Table 3). First, for some inhibitors the classification is essentially based upon inferences that need to be experimentally tested. Secondly, several inhibitors besides rotenone and piericidin A could interact at multiple sites depending on their concentration. Consequently, binding displacement results could over-estimate the extent of mutual overlapping in the specific interaction of certain inhibitors. However, by combining the results of complementary approaches it should be possible to define the most specific action of any inhibitor of complex I according to the three fundamental types outlined in Table 3.

Acknowledgements

I gratefully acknowledge the following colleagues who have participated to my research on complex I in the last few years: A. Ghelli, M. Ratta, F. Sparla, L. Bucchi, A. Andreani and V. Carelli (University of Bologna), A. Ngo, Q. Luu, M. Chow, G. Devlin (Monash University) and H. McLennan (University of Queensland). Without the dedication and patience of Anna, in particular, this work would have not been possible. I thank P. Bollinger of Sandoz and N. Rose of Novartis for the generous gifts of synthetic compounds.

References

[1] J. Walker, Q. Rev. Biochem. 25 (1992) 253–324.
[2] T. Friedrich, K. Steinmuller, H. Weiss, FEBS Lett. 367 (1995) 107–111.
[3] U. Brandt, Biochim. Biophys. Acta 1318 (1997) 79–91.
[4] T.P. Singer, R.R. Ramsay, in: L. Ernster (Ed.), Molecular Mechanisms in Bioenergetics, Elsevier, Amsterdam, 1992, pp. 145–162.
[5] R.M. Hollingworth, K.I. Ahammadsahib, G. Gadelhak, J.L. McLaughlin, Biochem. Soc. Trans. 22 (1994) 230–233.

[6] P. Jewess, Biochem. Soc. Trans. 22 (1994) 247–251.
[7] T. Friedrich, P. Van Heek, H. Leif, T. Ohnishi, E. Forche, B. Kunze, R. Jansen, W. Trowitzsch-Kienast, G. Hofle, H. Reichenbach, H. Weiss, Eur. J. Biochem. 219 (1994) 691–698.
[8] M. Degli Esposti, A. Ghelli, M. Ratta, D. Cortes, E. Estornell, Biochem. J. 301 (1994) 161–167.
[9] T.P. Singer, Meth. Enzymol. 55 (1979) 454–462.
[10] B.T. Stoner, Pharmacol. Ther. 10 (1980) 399–406.
[11] D.J. Horgan, H. Ohno, T.P. Singer, J.E. Casida, J. Biol. Chem. 243 (1968) 5967–5976.
[12] J. Burgos, E.R. Redfearn, Biochim. Biophys. Acta 110 (1965) 475–483.
[13] M. Gutman, T.P. Singer, J.E. Casida, J. Biol. Chem. 245 (1970) 1992–1997.
[14] M. Gutman, S. Kliatchko, FEBS Lett. 67 (1976) 348–353.
[15] K.H. Chung, K.Y. Cho, Y. Asami, N. Takahashi, S. Yoshida, Z. Naturforsch. 44c (1989) 609–616.
[16] M. Degli Esposti, A. Ghelli, M. Crimi, E. Estornell, R. Fato, G. Lenaz, Biochim. Biophys. Res. Commun. 190 (1993) 1090–1096.
[17] M. Degli Esposti, M. Crimi, A. Ghelli, Biochem. Soc. Trans. 22 (1994) 209–213.
[18] C. Lim, H. Ueno, H. Miyoshi, H. Miyagawa, H. Iwamura, T. Ueno, J. Pest. Sci. 21 (1996) 213–215.
[19] M. Enger, T. Anke, O. Sterner, U. Brandt, J. Antibiotics 50 (1997) 325–329.
[20] Y. Shimomura, T. Kawada, M. Suzuki, Arch. Biochem. Biophys. 270 (1989) 573–577.
[21] T. Yagi, Arch. Biochem. Biophys. 281 (1990) 305–311.
[22] T. Satoh, H. Miyoshi, K. Sakamoto, H. Iwamura, Biochim. Biophys. Acta 1273 (1996) 21–30.
[23] E.A. Kean, M. Gutman, T.P. Singer, J. Biol. Chem. 246 (1971) 2346–2353.
[24] N. Morikawa, Y. Nakagawa-Hattori, Y. Mizuno, J. Neurochem. 66 (1996) 1174–1181.
[25] M. Degli Esposti, A. Ngo, G. McMullen, A. Ghelli, F. Sparla, B. Benelli, M. Ratta, A.W. Linnane, Biochem. J. 313 (1996) 327–334.
[26] G. Lenaz, G.D. Daves, K. Folkers, Arch. Biochem. Biophys. 123 (1968) 539–550.
[27] R. Fato, E. Estornell, S. Di Bernardo, F. Pallotti, G. Parenti-Castelli, G. Lenaz, Biochemistry 35 (1996) 2705–2716.
[28] M. DegliEsposti, A. Ngo, A. Ghelli, B. Benelli, V. Carelli, H. McLennan, A.W. Linnane, Arch. Biochem. Biophys. 330 (1996) 395–400.
[29] B. Gassner, A. Wuthrich, G. Scholtysik, M. Solioz, J. Pharm. Exp. Ther. 281 (1997) 855–860.
[30] D.L. Gil, J. Ferreira, Xenobiotica 10 (1980) 7–15.
[31] A. Andreani, M. Rambaldi, A. Leoni, A. Locatelli, A. Ghelli, M. Ratta, B. Benelli, M. Degli Esposti, J. Med. Chem. 38 (1995) 1090–1097.
[32] L. Ernster, O. Jalling, O. Lindberg, Exp. Cell. Res. 3 (1955) 124–132.
[33] M. Filser, S. Werner, Biochem. Pharmacol. 37 (1988) 2551–2558.
[34] W.J. Nicklas, I. Vyas, R.E. Heikkila, Life Sci. 36 (1985) 2503–2508.
[35] L.M. Sayre, M.P. Singh, P.K. Arora, F. Wang, J.R. McPeak, C.L. Hoppel, Arch. Biochem. Biophys. 280 (1990) 274–283.
[36] M.R. Gluck, M.J. Krueger, R.R. Ramsay, S.O. Sablin, T.P. Singer, W.J. Nicklas, J. Biol. Chem. 269 (1994) 3167–3174.
[37] H. Miyoshi, M. Inoue, S. Okamoto, M. Ohshima, K. Sakamoto, H. Iwamura, J. Biol. Chem. 272 (1997) 16176–16183.
[38] R. Albores, E.J. Neafsey, G. Drucker, J.Z. Fields, M.A. Collins, Proc. Natl. Acad. Sci. U.S.A. 87 (1990) 9368–9372.
[39] L. Pecci, G. Montefoschi, M. Fontana, D. Cavallini, Biochem. Biophys. Res. Commun. 199 (1994) 755–760.
[40] K. Suzuki, Y. Mizuno, M. Yoshida, Neurochem. Res. 15 (1990) 705–710.
[41] C. Burkhardt, J.P. Kelly, Y. Lim, C.M. Filley, W.D.J. Parker, Ann. Neurol. 33 (1993) 512–517.
[42] H.R. McLennan, M. Degli Esposti, D.W. Eyles, J.J. McGrath, S.M. Pond, Mol. Pharmacol. (1997) (submitted).
[43] W.M. Anderson, H. Patheja, D. Delinck, W.W. Baldwin, S.T. Smiley, L.B. Chen, Biochem. Int. 19 (1989) 673–685.
[44] K. Veitch, L. Hue, Mol. Pharmacol. 45 (1994) 158–163.
[45] K.M. Wyatt, C. Skene, K. Veitch, L. Hue, J.G. McCormach, Biochem. Pharmacol. 50 (1995) 1599–1606.
[46] M. Degli Esposti, A. Ngo, M.A. Myers, Diabetes 45 (1996) 1531–1534.
[47] A.K. Tan, R.R. Ramsay, T.P. Singer, H. Miyoshi, J. Biol. Chem. 268 (1993) 19328–19333.
[48] W. Oettmeier, K. Masson, M. Soll, Biochim. Biophys. Acta 1099 (1992) 262–266.
[49] E. Reil, G. Hofle, W. Draber, W. Oettmeier, Biochim. Biophys. Acta 1318 (1997) 291–298.
[50] W.M. Anderson, D. Trgovcich-Zacok, Biochim. Biophys. Acta 1230 (1995) 186–193.
[51] A. Ghelli, B. Benelli, M. Degli Esposti, J. Biochem. 121 (1997) 746–755.
[52] C.I. Ragan, D.P. Bloxham, Biochem. J. 163 (1977) 605–615.
[53] T. Yagi, Biochemistry 26 (1987) 2822–2828.
[54] M. Finel, A. Majander, FEBS Lett. 339 (1994) 142–146.
[55] A. Majander, M. Finel, M. Wikstrom, J. Biol. Chem. 269 (1994) 21037–21042.
[56] T.P. Singer, M. Gutman, Adv. Enzymol. 34 (1971) 79–153.
[57] A.D. Vinogradov, J. Bioenerg. Biomembr. 25 (1993) 367–375.
[58] M. Degli Esposti, A. Ghelli, Biochim. Biophys. Acta 1187 (1994) 116–120.
[59] T. Cremona, E.B. Kearney, J. Biol. Chem. 240 (1965) 3645–3652.
[60] L. Ernster, C.P. Lee, Meth. Enzymol. 10 (1967) 729–738.
[61] P.C. Hinkle, R.A. Butow, E. Racker, J. Biol. Chem. 242 (1967) 5169–5173.
[62] G.A. Blondin, L.M. Knobeloch, H.W. Read, J.M. Harkin, Bull. Environ. Contam. Toxicol. 38 (1987) 467–474.

[63] Y. Hatefi, K.E. Stempel, W.G. Hanstein, J. Biol. Chem. 244 (1969) 2358–2365.
[64] F.J. Ruzicka, F.L. Crane, Biochim. Biophys. Acta 223 (1970) 71–85.
[65] Y. Hatefi, A.G. Haavik, D.E. Griffiths, J. Biol. Chem. 237 (1962) 1676–1680.
[66] E. Estornell, R. Fato, F. Pallotti, G. Lenaz, FEBS Lett. 332 (1993) 127–131.
[67] L. Helfenbaum, A. Ngo, A. Ghelli, A.W. Linnane, M. Degli Esposti, J. Bioenerg. Biomembr. 29 (1997) 71–80.
[68] V.G. Grivennikova, E.O. Malklashina, E.V. Gavrikova, A.D. Vinogradov, Biochim. Biophys. Acta 1319 (1997) 223–232.
[69] P.E. Lindhal, K.E. Oberg, Exp. Cell. Res. 23 (1961) 228–237.
[70] P. Ravanel, M. Tissut, R. Douce, Plant Physiol. 75 (1984) 414–420.
[71] E. Darrouzet, A. Dupuis, Biochim. Biophys. Acta 1317 (1997) 1–4.
[72] M.K. Phillips, D.B. Kell, FEBS Lett. 104 (1982) 248–250.
[73] K. Sakamoto, H. Miyoshi, K. Matsushita, M. Nakagawa, J. Ikeda, M. Ohshima, O. Adachi, T. Akagi, H. Iwamura, Eur. J. Biochem. 237 (1996) 128–135.
[74] D.C. Wallace, Science 256 (1992) 628–632.
[75] M. Erecinska, J. Bryla, M. Michalik, M.D. Meglasson, D. Nelson, Biochim. Biophys. Acta 1101 (1992) 273–295.
[76] R. Luft, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 8731–8738.
[77] K.I. Ahammadsahib, R.M. Hollingworth, J.P. McGovern, Y.-H. Hui, J.L. McLaughlin, Life Sci. 53 (1993) 1113–1120.
[78] R. Uslu, B. Bonavida, Cancer 77 (1996) 725–732.
[79] A. Cave, B. Figadere, A. Laurens, D. Cortes, in: W. Herz, G.W. Kirby, R.E. Moore, W. Steglich, C. Tamm (Eds.), Progress in the Chemistry of Organic Natural Products, Vol. 70, Springer-Verlag, Wien, 1997, pp. 21–288.
[80] H. Miyoshi, Biochim. Biophys. Acta (1998) (this issue).
[81] L. Ernster, G. Dallner, G.F. Azzone, J. Biol. Chem. 238 (1963) 1124–1131.
[82] D.J. Horgan, T.P. Singer, J.E. Casida, J. Biol. Chem. 243 (1968) 834–843.
[83] H. Heinrich, S. Werner, Biochemistry 31 (1993) 11413–11419.
[84] I. Ahmed, G. Krishnamoorthy, FEBS Lett. 300 (1992) 275–278.
[85] D.S.J. Higgins, J.T. Greenamyre, J. Neurosci. 16 (1996) 3807–3816.
[86] F.G.P. Earley, C.I. Ragan, Biochem. J. 224 (1984) 525–534.
[87] F.G.P. Earley, S.D. Patel, C.I. Ragan, G. Attardi, FEBS Lett. 219 (1987) 108–113.
[88] A. Majander, M. Finel, M.L. Savontaus, E. Nikoskelainen, M. Wikstrom, Eur. J. Biochem. 239 (1996) 201–207.
[89] M. Degli Esposti, V. Carelli, A. Ghelli, M. Ratta, M. Crimi, S. Sangiorgi, P. Montagna, G. Lenaz, E. Lugaresi, P. Cortelli, FEBS Lett. 352 (1994) 375–379.
[90] N. Takahashi, A. Suzuki, S. Tamura, J. Am. Chem. Soc. 87 (1965) 2066–2068.
[91] M. Gutman, C.J. Coles, T.P. Singer, J.E. Casida, Biochemistry 10 (1971) 2036–2043.
[92] S. Yoshida, Y. Nagao, A. Watanabe, N. Takahashi, Agric. Biol. Chem. 44 (1982) 2921–2924.
[93] M. Jeng, C. Hall, F.L. Crane, N. Takahashi, S. Tamura, K. Folkers, Biochemistry 7 (1968) 1311–1322.
[94] M. Gutman, Biochim. Biophys. Acta 594 (1980) 53–84.
[95] R.R. Ramsay, M.J. Krueger, S.K. Youngster, T.P. Singer, Biochem. J. 273 (1991) 481–484.
[96] M.C. Zafra-Polo, M.C. Gonzalez, E. Estornell, S. Sahpaz, D. Cortes, Phytochemistry 42 (1996) 253–271.
[97] M. Londerhausen, W. Leicht, F. Lieb, H. Moeschler, H. Weiss, Pest. Sci. 33 (1991) 427–438.
[98] M.C. Gonzales, J.R. Tormo, A. Bermejo, M.C. Zafra-Polo, E. Estornell, D. Cortes, Bioorg. Med. Chem. Lett. 7 (1997) 1113–1118.
[99] V. Carelli, A. Ghelli, M. Ratta, E. Bacchilega, S. Sangiorgi, R. Mancini, V. Leuzzi, P. Cortelli, P. Montagna, E. Lugaresi, M. Degli Esposti, Neurology 48 (1997) 1623–1632.
[100] H. Reichenbach, K. Gerth, H. Irschik, B. Kunze, G. Hofle, Trends Biotechnol. 6 (1988) 115–121.
[101] K. Gerth, R. Jansen, G. Reifenstahl, G. Hofle, H. Irschik, B. Kunze, H. Reichenbach, G. Thierbach, J. Antibiotics 36 (1983) 1150–1156.
[102] W. Trowitzsch-Kienast, E. Forche, V. Wray, H. Reichenbach, E. Jurkiewicz, G. Hunsmann, G. Hofle, Liebigs Ann. Chem. 1992 (1992) 659–664.
[103] R. Janzen, B. Kunze, H. Wray, H. Reichenbach, E. Jurkiewicz, G. Hunsmann, G. Hofle, Liebigs Ann. Chem. 1991 (1991) 707–708.
[104] R. Janzen, B. Kunze, H. Reichenbach, E. Jurkiewicz, G. Hunsmann, G. Hofle, Liebigs Ann. Chem. 1992 (1991) 357–359.
[105] B. Meunier, S.A. Madgwick, E. Reil, W. Oettmeier, P.R. Rich, Biochemistry 34 (1995) 1076–1083.
[106] Y. Hirata, H. Nakata, K. Yamada, K. Okuhara, T. Naito, Tetrahedron 14 (1961) 252–274.
[107] M.J. Caterina, M.A. Schumacher, M. Tominaga, T.A. Rosen, J.D. Levine, D. Julius, Nature 389 (1997) 816–824.
[108] J. Szolcsanti, A. Jancso-Gabor, Nature 229 (1971) 116–117.
[109] I. Imada, T. Fujita, Y. Sugiyama, K. Okamato, Y. Kobayashi, Arch. Gerontol. Geriatr. 8 (1989) 323–341.
[110] C. Longhurst, L. Bacci, J. Buendia, C.J. Hatton, J. Petitprez, P. Tsakonas, Pests and diseases, Brighton Crop Protection Conference, British Crop Protection Council, 1992, pp. 51–58.
[111] B. Latli, E. Wood, J.E. Casida, Chem. Res. Toxicol. 9 (1996) 445–450.
[112] J.G. Okun, H. Schagger, U. Brandt, Ninth EBEC Reports, 1996, p. 147.
[113] K. Motoba, T. Suzuki, M. Uchida, Pest. Biochem. Physiol. 43 (1992) 37–44.

[114] K. Hirata, Y. Kawamura, M. Kudo, H. Igarashi, J. Pest. Sci. 20 (1995) 177–179.
[115] H. Saito, M. Hirano, T. Shigeoka, J. Pest. Sci. 19 (1994) 93–101.
[116] W.G. Thwaite, C.C. Bower, A.M. Hately, D. Swist-Swirski, Exp. Appl. Acarol. 20 (1996) 177–191.
[117] G. Herron, L. Jiang, R. Spooner-Hart, Exp. Appl. Acarol. 20 (1996) 495–502.
[118] E. Kuwano, N. Sato, M. Eto, Agric. Biol. Chem. 46 (1982) 1715–1716.
[119] E. Cohen, E. Kuwano, M. Eto, Agric. Biol. Chem. 48 (1984) 1617–1620.
[120] Y. Nakagawa, E. Kuwano, M. Eto, T. Fujita, Agric. Biol. Chem. 49 (1985) 3569–3573.
[121] A. Andreani, M. Rambaldi, A. Locatelli, F. Andreani, G. Poglayen, M. Degli Esposti, Eur. J. Med. Chem. 27 (1992) 729–734.
[122] T.P. Singer, A.J. Trevor, N.J. Castagnoli, TIBS 12 (1987) 266–270.
[123] S. Werner, Biochem. Pharmacol. 38 (1989) 1807–1818.
[124] M.J. Krueger, T.P. Singer, J.E. Casida, R.R. Ramsay, Biochem. Biophys. Res. Commun. 169 (1990) 123–128.
[125] R. Ramsay, M.J. Krueger, S.K. Youngster, M.R. Gluck, J.E. Casida, T.P. Singer, J. Neurochem. 56 (1991) 1184–1190.
[126] M. Naoi, W. Maruyama, Eur. Neurol. 3 (1993) 31–37.
[127] D. Ben-Sachar, R. Zuk, Y.Y. Glinka, J. Neurochem. 64 (1995) 718–723.
[128] Y.Y. Glinka, M.B.H. Youdim, Eur. J. Pharmacol. 292 (1995) 329–332.
[129] K.F. Gey, M. Rutishauser, A. Pletscher, Biochem. Pharmacol. 14 (1965) 507–514.
[130] J.Z. Byczkowski, R. Borysewicz, Gen. Pharmacol. 10 (1979) 369–372.
[131] C.D. Marsden, P. Jenner, Psychol. Med. 10 (1980) 55–72.
[132] B. Subramanyam, H. Rollema, T. Woolf, N.J. Castagnoli, Biochem. Biophys. Res. Commun. 166 (1990) 238–244.
[133] D.W. Eyles, H.R. McLennan, A. Jones, J.J. McGrath, T.J. Stedman, S.M. Pond, Clin. Pharmacol. Ther. 56 (1994) 512–520.
[134] J.H. Karam, P.A. Lewitt, C.W. Young, R.E. Nowlain, B.J. Frankel, H. Fujiya, Z.R. Freedman, G.M. Grodsky, Diabetes 29 (1980) 971–978.
[135] W. Oettmeier, K. Masson, E. Reil, Biochem. Soc. Trans. 22 (1994) 213–216.
[136] A.S. Waggoner, Ann. Rev. Biophys. Bioeng. 8 (1979) 47–68.
[137] W.M. Anderson, D.L. Delinck, L. Benninger, J.M. Wood, S.T. Smiley, L.B. Chen, Biochem. Pharmacol. 45 (1993) 91–696.
[138] W.M. Anderson, J.M. Wood, A.C. Anderson, Biochem. Pharmacol. 45 (1993) 2115–2122.
[139] I.I. Ahmed, G. Krishnamoorthy, Biochim. Biophys. Acta 1188 (1994) 131–138.
[140] H.J. Harmon, F.L. Crane, Biochim. Biophys. Acta 226 (1976) 221–233.
[141] A.B. Kotlyar, M. Gutman, Biochim. Biophys. Acta 1140 (1992) 169–174.
[142] H. Suzuki, T.E. King, J. Biol. Chem. 258 (1983) 352–358.
[143] A.B. Kotlyar, V.D. Sled, D.S. Burbaev, I.A. Moroz, A.D. Vinogradov, FEBS Lett. 264 (1990) 17–20.
[144] R. Van Balzen, A.B. Kotlyar, N. Moon, W.R. Dunham, S.P.J. Albracht, Biochemistry 36 (1997) 886–893.