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EFFECTS OF ANTI-INFLAMMATORY 2-ARYL-1,3-INDANDIONES ON OXIDATIVE PHOSPHORYLATION IN RAT LIVER MITOCHONDRIA

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Abstract—A number of 2-aryl-1,3-indandiones were examined for action on oxidative phosphorylation in rat liver mitochondria. They were all found to be uncouplers and to stimulate state 4 respiration and mitochondrial ATPase and to reduce the P/O ratio. There exists a marked difference in activity between 2-aryl-1,3-indandiones with and without ortho substituents. The latter have the same properties as 2,4-dinitrophenol. Uncoupling activity is dependent on the presence of an acid proton but lipophilicity also plays a major role. In high concentrations, all 2-aryl-1,3-indandiones can inhibit respiration but with the ortho-substituted compounds inhibition is most overt. The anti-inflammatory activities of 2-aryl-1,3-indandiones, as determined in a carrageenan oedema test, showed no relationship to uncoupling of oxidative phosphorylation.

The group of compounds, usually indicated as anti-inflammatory drugs, act through a mechanism which, because of the complicated nature of inflammation, has not been fully elucidated yet. In recent years attempts have been made to obtain more insight into this problem by studying the influence of a large variety of anti-inflammatory agents on biochemical processes [1–3]. It is remarkable that although anti-inflammatory drugs form a very heterogeneous group, they have many biochemical properties in common, one of which is the uncoupling of oxidative phosphorylation. The relationship between uncoupling and anti-inflammatory activity was first studied by Adams and Cobb [4] and then in greater detail by Whitehouse [1, 5, 6].

In 1961 Fontaine *et al.* [7] reported 2-phenyl-1,3-indandione (PID) to have not only anticoagulant but also anti-inflammatory properties. Many derivatives have since been tested for the latter activity [8, 9], but so far no useful anti-inflammatory drugs have been discovered along these lines. It should be noted here that PID has been found to uncouple oxidative phosphorylation both *in vitro* [10] and *in vivo* [11].

The present work describes the effects of a series of 2-aryl-1,3-indandiones on the respiration, oxidative phosphorylation and ATPase activity in rat liver mitochondria. For a number of these compounds, the anti-inflammatory activity was evaluated in order to establish any correlation with uncoupling of oxidative phosphorylation.

MATERIALS AND METHODS

Special chemicals. Most of the 2-aryl-1,3-indandiones were synthesized in our laboratories using the methods described in the literature [12, 13]. Compounds 46 and 64 (see Tables 2 and 4, respectively) were gifts from Gist-Brocades, Delft, Netherlands, and compounds 29, 30, 31, 38–44 and 47–51 (see Tables 1, 2 and 3) were obtained from Dr. J. A. Durden Jr, Union Carbide, South Charleston, U.S.A.

2,4-Dinitrophenol (DNP) was obtained from British Drug Houses, salicylic acid from E. Merck AG and phenylbutazone from Geigy-Ciba. Indomethacin was kindly supplied by Merck, Sharpe & Dohme, Nederland B.V., and 55 (see Table 3) was synthesized according to the method of Horiuchi *et al.* [14].

ATP and hexokinase (Type III) were obtained from Sigma Chemical Co. and Carrageenan (Viscarin 402, lot No. 450602) from Marine Colloids Inc.

All other chemicals were analytical grade preparations from the usual sources.

Preparation of the mitochondria. Rat liver mitochondria were isolated from male albino rats (Wistar strain) weighing 200–250 g, according to the method of Myers and Slater [15] with some modifications. The liver homogenate was centrifuged at 900 *g* for 10 min and the supernatant was recentrifuged at 5000 *g* for 15 min. The mitochondrial pellet was washed by resuspension in 0·25 M sucrose and centrifugation at 14,000 *g* for 10 min. Protein content was determined by the method of Lowry *et al.* [16].

Respiration and oxidative phosphorylation. Respiration and oxidative phosphorylation were measured manometrically as described by Slater [17] at 25° in a medium (3 ml) containing 32 mM sucrose, 15 mM KCl, 5 mM $MgCl_2$, 2 mM EDTA, 50 mM Tris–HCl, 16·7 mM potassium phosphate, 8 mM Na glutamate, 0·9 mM ATP, 16·7 mM glucose, 7 units of hexokinase and about 5 mg of mitochondrial protein. The final pH was 7·5. Drugs were added in an ethanolic solution of 0·1 ml. The reaction was stopped after 20 min by addition of 0·5 ml of 40% (w/v) trichloroacetic acid. The precipitate was centrifuged and inorganic phosphate in the supernatant was assayed by the method of Traussky and Shorr [18]. The oxygen uptake in the absence of ADP was measured in the same medium but hexokinase and ATP were omitted.

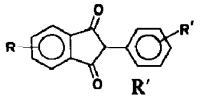
ATPase activity. ATPase activity was measured as described by Hemker [19] in a medium (1·5 ml) containing 50 mM sucrose, 75 mM KCl, 50 mM Tris–HCl, 1 mM EDTA, 3 mM $MgCl_2$, 2 mM ATP and about 0·2 mg of mitochondrial protein. The final pH was 7·5. Drugs were added in an ethanolic solution of 0·05 ml. The reaction was carried out at 25° for 10 min, and stopped by addition of 0·5 ml of 10% (w/v) trichloroa-

Table 1. Effects of 2-(R-phenyl)-1,3-indandiones on respiration, oxidative phosphorylation and ATPase activity in rat liver mitochondria

No.	Derivative	log $1/c_{50}$ (M^{-1})	resp.$_{50}$ (%)	log $1/cr_{50}$ (M^{-1})	log $1/c_m$ (M^{-1})	log $1/c_{\frac{1}{2}m}$ (M^{-1})	max (%)
1	Unsubstituted	3·74	100	3·23	3·69	4·04	100
2	4-Methyl	4·10	98	—	4·10	4·48	103
3	4-Ethyl	4·53	104	3·49	4·51	4·87	109
4	4-Isopropyl	4·87	101	—	4·80	5·23	113
5	4-*t*-Butyl	5·03	102	3·69	5·03	5·43	102
6	4-*n*-Octyl	5·66	106	—	6·25	6·58	107
7	4-Phenyl	4·98	104	—	5·28	5·62	102
8	4-Chloro	4·20	101	—	4·27	4·50	86
9	4-Bromo	4·31	103	3·72	4·24	4·62	83
10	4-Trifluoromethyl	4·11	97	—	4·12	4·36	74
11	4-Methoxy	3·75	94	—	3·75	4·07	96
12	3-Methyl	4·01	105	3·37	4·05	4·46	91
13	3-Ethyl	4·42	104	—	4·46	4·84	92
14	3-Isopropyl	4·69	105	3·65	4·69	5·07	103
15	3-*t*-Butyl	4·95	101	—	4·90	5·21	95
16	3-Chloro	4·02	97	3·77	4·28	4·50	98
17	3-Trifluoromethyl	3·99	106	—	4·11	4·41	81
18	3-Methoxy	3·62	100	—	3·77	3·95	88
19	3,5-Dimethyl	4·42	107	3·61	4·43	4·76	103
20	3,5-Diethyl	5·06	106	—	4·97	5·34	104
21	3,5-Diisopropyl	5·41	103	—	5·46	5·81	119
22	3,5-Di-*t*-butyl	5·61	100	5·05	5·77	6·27	108
23	3,5-Dichloro	4·31	89	4·14	4·85	5·08	68
24	3,5-Dimethoxy	3·38	100	—	3·67	3·90	76
25	2-Methyl	3·46	74	3·27	—	3·93	76
26	2-Ethyl	3·65	70	3·40	—	4·03	87
27	2-Isopropyl	3·92	90	—	—	4·44	90
28	2-*t*-Butyl	3·66	78	—	—	4·17	75
29	2-Fluoro	3·25	86	—	—	3·84	65
30	2-Chloro	3·28	66	3·12	—	3·91	60
31	2-Bromo	3·31	47	3·33	—	3·88	55
32	2-Trifluoromethyl	3·08	40	—	—	3·65	54
33	2,6-Dimethyl	3·45	40	3·62	—	3·80	65
34	2,6-Diethyl	3·77	62	3·58	—	4·19	51
35	2,6-Diisopropyl	4·11	105	—	—	4·58	101
36	2-Methyl-6-ethyl	3·65	50	3·65	—	4·05	53
37	2-Methyl-6-isopropyl	3·93	57	3·88	—	4·28	72
38	2,6-Dichloro	3·11	30	3·36	—	3·58	36
39	2-Methyl-6-chloro	3·32	22	3·53	—	3·79	47
40	2,4-Dimethyl	3·58	89	—	—	4·11	76
41	2,5-Dimethyl	3·59	88	—	—	4·07	79
42	2,4,6-Trimethyl	3·60	86	3·24	—	4·00	65
43	2,4,6-Trichloro	3·52	58	3·44	—	3·99	80
44	2,6-Dimethyl-4-*t*-butyl	4·39	102	—	—	4·73	100

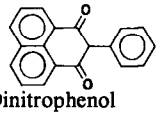
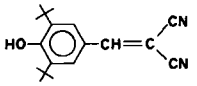
c_{50}: Concn for 50% uncoupling (50% reduction in P/O ratio). resp.$_{50}$: Respiration rate in the presence of phosphate acceptor at 50% uncoupling as % of control. cr_{50}: Concn for 50% inhibition of respiration. c_m: Concn for maximal ATPase activity. $c_{\frac{1}{2}m}$: Concn for half-maximal stimulation of ATPase activity. max: Maximal ATPase activity as % of the maximal value for PID.

Table 2. Effects of 2-phenyl-1,3-indandione derivatives on respiration, oxidative phosphorylation and ATPase activity in rat liver mitochondria

No.	 R	R′	log $1/c_{50}$ (M^{-1})	resp.$_{50}$ (%)	log $1/c_m$ (M^{-1})	log $1/c_{\frac{1}{2}m}$ (M^{-1})	max (%)
45	5-*t*-Butyl	H	4·95	103	5·01	5·35	108
46	5-Trifluoro-methyl	3-methyl	4·38	111	4·77	5·01	101
47	5,6-Dimethyl	2,4,6-trimethyl	3·83	93	—	4·32	79
48	5,6-Dimethyl	2,6-dichloro	3·42	69	—	4·05	51
49	4-Methyl	2,6-dichloro	3·75	33	—	4·32	41
50	4,7-Dimethyl	2,6-dichloro	4·13	61	—	4·49	54

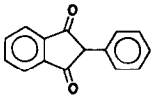
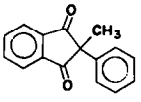
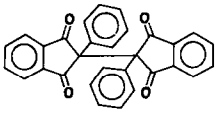
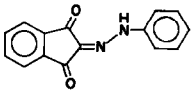
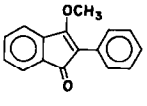
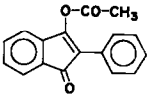
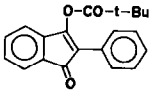
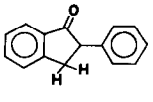
Notation as Table 1.

Table 3. Effects of various compounds on respiration, oxidative phosphorylation and ATPase activity in rat liver mitochondria

No.	Compound	log $1/c_{50}$ (M^{-1})	resp.$_{50}$ (%)	log $1/c_m$ (M^{-1})	log $1/c_{\frac{1}{2}m}$ (M^{-1})	max (%)
51	2-(1-Naphtyl)-1,3-indandione	3·86	78	—	4·38	76
52	2-(2-Naphtyl)-1,3-indandione	4·61	102	4·85	5·11	108
53		3·44	71	—	4·03	34
54	2,4-Dinitrophenol	4·73	106	4·19	—	107
55		7·70	—	—	—	—
56	Salicylic acid	2·62	—	—	3·44	48
57	Phenylbutazone	3·15	66	3·59	3·86	25
58	Indomethacin	3·54	40	—	4·20	29

Notation as Table 1.

Table 4. Effects of 2-phenyl-1,3-indandione and some derivatives on respiration and oxidative phosphorylation (in per cent of control)

No.	Compound	Concn (10^{-5} M)	Respiration −ADP	Respiration +ADP	P/O
1		3·3	120	100	90
		10	200	102	71
		20	450	103	40
59		20	100	100	100
60		3·3	100	100	100
61		10	132	75	100
62		10	67	70	100
63		10	163	51	53
64		10	NP	100	100
65		20	NP	65	100

NP—not performed.

cetic acid and the inorganic phosphate was assayed as indicated by Traussky and Shorr [18].

Anti-inflammatory activity. Anti-inflammatory activity was evaluated using the method of Winter *et al.* [20]. Groups of six male albino rats (Wistar strain), weighing 110–150 g, were used. They were fasted overnight but tap water was provided *ad lib.* The test compounds were administered orally as a suspension in 1% amylum 1 hr before the injection of carrageenan. Dose: 1 ml of suspension/100 g of body wt. The controls merely received the vehicle.

Hind paw oedema was produced by injecting 0·05 ml of 1% carrageenan in 0·9% saline into the plantar surface. Paw volume was measured before and 3 and 4 hr after injection using a plethysmograph as described by Lence [21].

RESULTS

Respiration and oxidative phosphorylation. A study was made of the effects of 2-aryl-1,3-indandiones on the P/O ratio and on the respiration rate in states 3 and 4 (according to the definitions of Chance and Williams [22]). On the basis of the results obtained, they may be divided into two groups, one of compounds with ortho-substituents and one of compounds without these substituents. PID and the meta- and para-substituted derivatives stimulate state 4 respiration, reduce the P/O ratio and do not inhibit respiration until their concentrations exceed those needed for maximal uncoupling. Their activities, therefore, are reminiscent to those of DNP [19, 23]. The ortho-substituted compounds do reduce the P/O ratio but inhibit respiration before maximal uncoupling has been attained. Because of this, stimulation of state 4 respiration is limited. Figure 1 shows the results for some compounds.

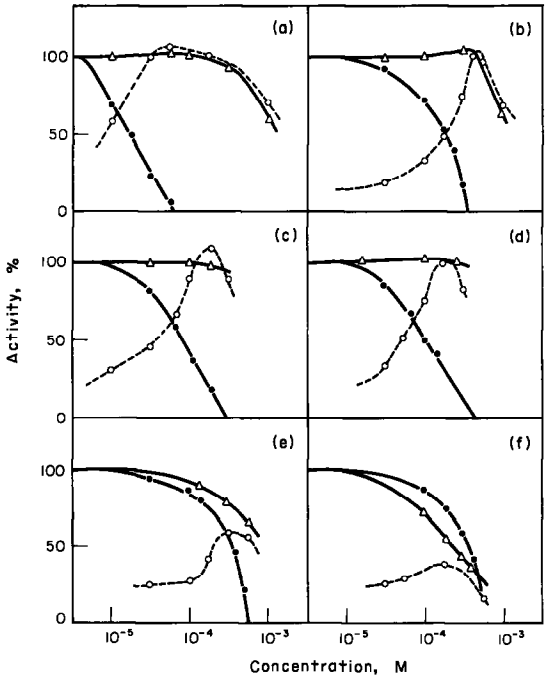


Fig. 1. Effects of 2,4-dinitrophenol (a) and some 2-(R-phenyl)-1,3-indandiones on respiration and phosphorylation. (b) R = H; (c) R = 4-methyl; (d) R = 3-methyl; (e) R = 2-methyl and (f) R = 2,6-dimethyl. (●) P/O ratio as % of control; (△) respiration in the presence of ADP and (○) respiration in the absence of ADP—as per cent of state 3 respiration.

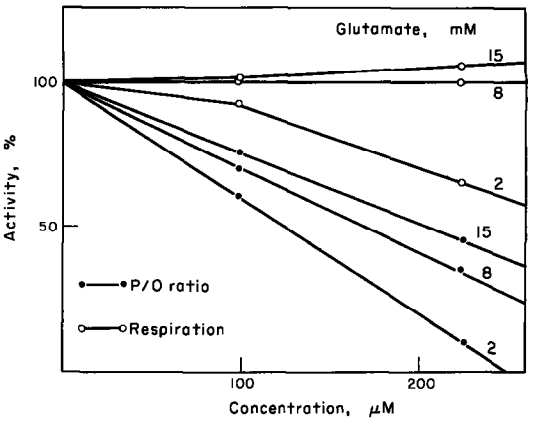


Fig. 2. Effects of 2-phenyl-1,3-indandione on respiration and P/O ratio at different substrate concentrations as per cent of control.

The activity of an uncoupling agent has been characterized by the concentration at which the P/O ratio is reduced to 50 per cent (c_{50}). The activities of the 2-aryl-1,3-indandiones and the respiration rate in the presence of phosphate acceptor at 50 per cent uncoupling as per cent of control value (resp.$_{50}$) are listed in Tables 1 and 2. All the figures recorded are the means of five to six experiments, the S.D. being 2–8 per cent.

Inhibition of respiration by ortho-substituted compounds was more pronounced; hence it was examined in more detail. For 22 compounds the concentration inhibiting state 3 respiration by 50 per cent, was determined (cr_{50} in Table 1).

Experiments carried out with a much lower substrate concentration showed that, under this condition, PID also inhibits respiration before maximal uncoupling has been reached (see Fig. 2). Inhibition of respiration by uncouplers is known to be kinetically competitive [24]. As can be seen in Fig. 3, this is also the case with a few 2-aryl-1,3-indandiones.

Table 3 shows the uncoupling activities of some related compounds, DNP, 3,5-di-*t*-butyl-4-hydroxybenzylidenemalononitrile (55) and a few anti-inflammatory therapeutics.

To establish on what structural elements of the PID molecule activity was dependent, the influence of some compounds chemically related to PID on the respiration in states 3 and 4 and the P/O ratio were studied. This was, however, merely possible in fairly low concentrations because of poor water solubility of these compounds. Table 4 shows the effects at the highest obtainable concentration as a per cent of the control value.

ATPase activity. As regards action on mitochondrial ATPase, 2-aryl-1,3-indandiones also fall into two categories. PID and its meta- and para-substituted derivatives stimulate ATPase activity and in common with the corresponding curve of DNP [19], the concentration–activity curve displays a clear-cut maximum. The ortho-substituted compounds, too, stimulate ATPase activity to a maximal value which is, however, mostly lower than those of the meta and para compounds. With the ortho-substituted compounds

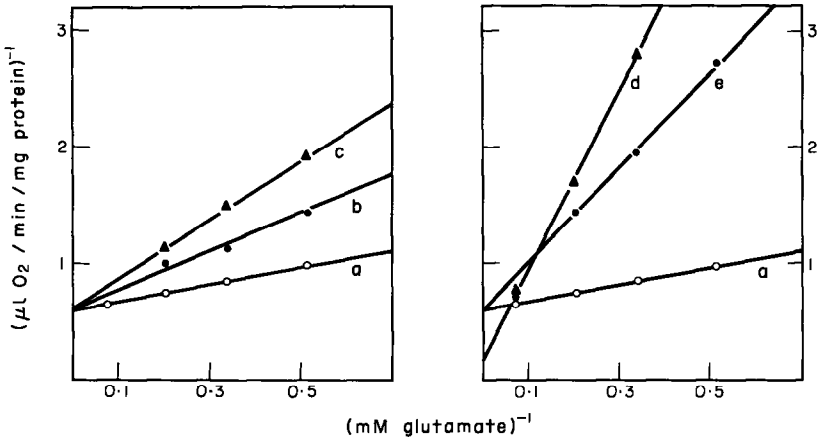


Fig. 3. Lineweaver–Burk plot of the inhibition of glutamate oxidation by uncoupling agents. (a) control, (b) DNP ($3{\cdot}3 \times 10^{-4}$ M), (c) PID (5×10^{-4} M), (d) 2-(2-methylphenyl)-1,3-indandione (4×10^{-4} M) and (e) 2-(2,6-dimethylphenyl)-1,3-indandione ($1{\cdot}7 \times 10^{-4}$ M).

inhibition only occurs at much higher concentrations, so that a broad plateau is formed, as is illustrated in Fig. 4.

The ATPase activities of the 2-aryl-1,3-indandiones are represented by three parameters in Tables 1–3, where c_m = the concentration at which maximal activity is reached [this concentration could only be determined accurately in the case of a well-defined maximum (non-ortho compounds)], $c_{\frac{1}{2}m}$ = the concentration at which stimulation is half-maximal and max = maximal ATPase activity as per cent of the maximum for PID. The results are the means of five experiments, the S.D. ranging from 10 to 20 per cent.

Anti-inflammatory activity. Injection of carrageenan into the hind paw of the rat caused an oedema of $0{\cdot}65 \pm 0{\cdot}10$ ml (at 3 hr) and $0{\cdot}69 \pm 0{\cdot}09$ ml (at 4 hr). These figures ($\pm$S.D.) are the means of 84 rats. Swelling was maximal at 4 hr after carrageenan.

The compounds were tested in doses of 5, 25 and 100 mg/kg, and only the most active ones in a dose of 1 mg/kg. Maximal inhibition was observed at 3 or 4 hr after the injection of carrageenan, depending on the pharmaco-kinetical properties of the compounds whereas the effects were less in an earlier stage.

Each value shown in Table 5 is the mean of the inhibitions at 3 and 4 hr after carrageenan, and these were used to determine graphically the concentration at which 50 per cent inhibition occurs (ED_{50}).

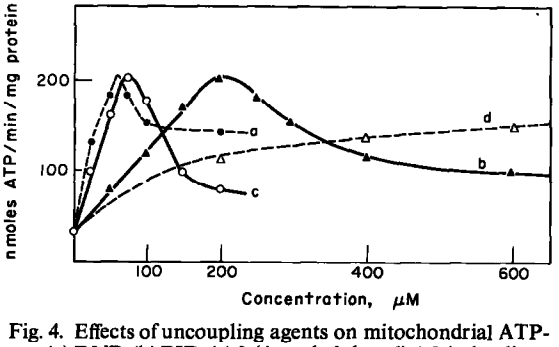


Fig. 4. Effects of uncoupling agents on mitochondrial ATPase. (a) DNP, (b) PID, (c) 2-(4-methylphenyl)-1,3-indandione and (d) 2-(2-methylphenyl)-1,3-indandione.

DISCUSSION

Whitehouse and Leader [10] found PID to be an uncoupling agent and its 4-chloro derivative to be four times as active. This is in agreement with our results. All the 2-aryl-1,3-indandiones tested appear to be uncouplers, as they stimulate state 4 respiration and mitochondrial ATPase, reduce the P/O ratio and, in higher concentrations, inhibit respiration. Para and meta substitution enhance uncoupling activity (see Table 1). There is a remarkable agreement in activity between the para and meta series; 3, 13 and 19 are about equally active and so are, for example, 5, 15, 20 and 45 and 6 and 22. There is also a rise in activity as the number of carbon atoms in the substituents increase. A similar trend is noted in the ortho series but here the activity level is much lower. This indicates that lipophilicity is a major parameter and is in agreement with other structure-activity relationship studies [19, 25, 26]. Introduction of halogen substituents in ortho position has been found to lower activity considerably. The difference between ortho and non-ortho compounds, as clearly shown in Fig. 1 and Tables 1 and 2, resembles the difference found by Parker [27] between two groups of phenols. He provided no further explanation for this phenomena. A structure–activity relationship study using multiple regression analysis showed us [28] that in the case of 2-aryl-1,3-indandiones the difference not merely results from steric effects of the ortho-substituents, as the uncoupling activity was not dependent on E_s^o (steric parameter of Taft [29]). Reduction in P/O ratio and stimulation of ATPase activity correlate strikingly well, the correlation coefficient being 0·981 (44 compounds). This is comprehensible as both effects result from the same uncoupling reaction. In most cases, uncoupling agents inhibit respiration at concentrations much higher than those needed for 100 per cent uncoupling. As the ortho compounds are weak uncouplers, they produce inhibition of respiration and uncoupling at the same time, and even at 50 per cent uncoupling there is a markedly inhibited respiration (cf. $resp._{50}$ values in Tables 1–3). This is also seen on comparison of the $-\log c_{50}$ and $-\log cr_{50}$ values given in Table 1. Inhibition of respiration thus appears to be not related to uncoupling, an inference which is in accord-

Table 5. Anti-inflammatory activities of 2-(R-phenyl)-1,3-indandiones and some other compounds

No.	Derivative	Inhibition of oedema (%) at doses (mg/kg) of 1	5	25	100	ED_{50} (mg/kg)
1	Unsubstituted	9	11	44	67	37
3	4-Ethyl			20	73	56
5	4-*t*-Butyl		7	13	30	540
6	4-*n*-Octyl		22	20		—
7	4-Phenyl			0	42	131
8	4-Chloro		17	42	77	31
10	4-Trifluoromethyl		2	28	52	89
11	4-Methoxy		0	18	58	77
12	3-Methyl			18	62	70
15	3-*t*-Butyl		19	48	67	31
16	3-Chloro		3	31	51	93
17	3-Trifluoromethyl		0	23	56	78
18	3-Methoxy		0	15	40	177
20	3,5-Diethyl		21	30	59	65
21	3,5-Diisopropyl		18	29	44	170
22	3,5-Di-*t*-butyl	25	52	69		5·4
23	3,5-Dichloro			22		—
24	3,5-Dimethoxy		0	13	53	97
25	2-Methyl			8	50	100
26	2-Ethyl			0	5	—
28	2-*t*-Butyl			0	0	—
34	2,6-Diethyl			11	0	—
35	2,6-Diisopropyl			12	48	110
37	2-Methyl-6-isopropyl			0	10	—
45	5'-*t*-Butyl		22	39		76
51	2,3-$(CH)_4$			5	39	151
52	3,4-$(CH)_4$			0	51	97
54	2,4-Dinitrophenol		6	37		50
55	Malononitrile*		14	27		500
56	Salicylic acid			2	30	310
57	Phenylbutazone		13	43	60	46
58	Indomethacin	26	57			3·6

* 3,5-Di-*t*-butyl-4-hydroxybenzylidenemalononitrile.
The inhibition of oedema formation was the mean of the inhibition at 3 and 4 hr after carrageenan injection.

ance with the results of Wilson and Merz [24]. In the case of lower substrate concentrations, PID has likewise been found to inhibit respiration before uncoupling is 100 per cent, and inhibitions by PID and two ortho-substituted compounds are competitive (Fig. 3), so that it does not seem likely that inhibition by ortho-substituted compounds is based on another mechanism than inhibition by meta and para derivatives.

When the inhibition of respiration at 50 per cent uncoupling is compared with the maximum attainable ATPase activity, they appear to have some features in common. Both might be caused by inhibition of the accumulation of substrates (glutamate and ATP, respectively). Van Dam *et al.* [30, 31] have demonstrated the possibility of such an inhibition.

PID derivatives whose acidic properties were eliminated, proved inactive, which points out that uncoupling activity is dependent on the presence of an acid proton (see Table 4). 3-Acetoxy-2-phenylindone (63) does show activity but a check by using thin-layer chromatography demonstrated rapid hydrolysis to PID in the medium used. The relation between uncoupling activity and the presence of an acidic group has also been demonstrated for other types of uncouplers, such as phenols [32], phenylhydrazones [33] and benzimidazoles [34], and a mechanism of uncoupling by lipid-soluble weak acids has been proposed by Van Dam and Kraaijenhof [31, 35].

Much vaguer is the relationship between structure and anti-inflammatory activity of 2-aryl-1,3-indandiones. As can be seen in Table 5, PID and phenylbutazone are about equally active, which agrees with what has been found by Fontaine *et al.* [36]. In general, activity is lowered by substitution. Compounds 8, 15 and 45 are just as active as PID; only 22 is clearly more active and comparable to indomethacin. A tert-butyl group in meta position causes good activity (cf. 15, 22 and 45) but 6 and 21 have such a low activity that lipophilicity cannot be a decisive factor here. The values of the three anti-inflammatory reference compounds correspond to those reported by Winter *et al.* [20, 37].

Anti-inflammatory activity is plotted against uncoupling in Fig. 5. The correlation, as postulated by Whitehouse [1, 6], would appear to be applicable to salicylic acid, phenylbutazone and indomethacin. Most of the 2-aryl-1,3-indandiones are, however, more potent uncoupling agents than indomethacin whilst they are less anti-inflammatory than phenylbutazone. Within the indandione series the correlation is also absent. The most active uncoupler, 6, possesses hardly any anti-inflammatory activity whereas PID is weak uncoupling agent but has a good anti-

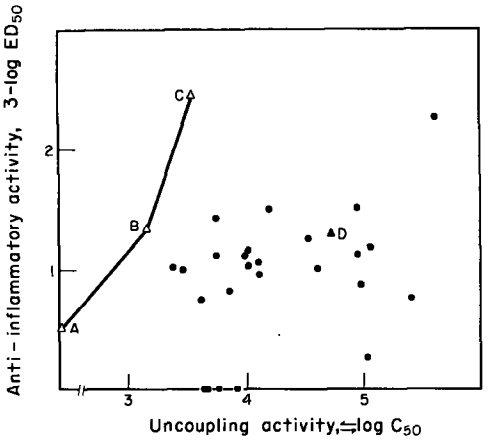


Fig. 5. Correlation between anti-inflammatory activity and uncoupling of oxidative phosphorylation by 2-aryl-1,3-indandiones (●), salicylic acid (A), phenylbutazone (B), indomethacin (C) and DNP (D).

inflammatory activity. Malononitrile 55, the strongest uncoupler known at this moment [38], is only a very poor anti-inflammatory agent. Saeki *et al.* [39] found no relationship between uncoupling and anti-inflammatory activity for anthranilic acid derivatives, which renders the validity of Whitehouse's hypothesis doubtful. It is, of course, conceivable that because of differences in metabolism, solubility, transport and excretion no correlation can be demonstrated but an obvious correlation between ATP-production and inflammation was also absent in other studies reported in the literature. Although significantly raised in inflamed tissue, ATP-levels cannot be lowered by anti-inflammatory drugs[40, 41]. In cell cultures anti-inflammatory drugs enhance ATP-levels [41, 42], in contrast to DNP, which lowers them. It should also be noted that the complexity of the inflammatory process may permit a multiple mechanism of action, in which case it is impossible to correlate simple *in vitro* tests with *in vivo* results.

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