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INFLUENCE OF NON-STEROID ANTI-INFLAMMATORY DRUGS (NSAIDs) ON HEPATIC TYROSINE AMINOTRANSFERASE (TA) ACTIVITY IN RATS *IN VITRO* AND *IN VIVO**†

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Abstract—Indomethacin, flufenamic acid, mefenamic acid, gold sodium thiosulphate and ibuprofen were found to be inhibitors of hepatic TA *in vitro*. The type of inhibition was competitive relative to L-tyrosine; apparent K_i values ranged from $0{\cdot}7 \times 10^{-4}$ to $1{\cdot}18 \times 10^{-3}$ M. Several other NSAIDs did not, or poorly, inhibit TA at concentrations of $\leq 10^{-3}$ M. In intact rats, TA activity was stimulated 18-fold by pretreatment with mefenamic acid, 7-fold by dimethylsulfoxide and ibuprofen, 5-fold by acetylsalicylic and flufenamic acid, and doubled by benzydamine, amidopyrine, sodium salicylate and gold sodium thiosulphate. Indomethacin was somewhat less effective. Drug dosage was 20 per cent of the acute p.o. LD_{50}, three times with 12-hourly intervals. Phenylbutazone, chloroquine and cyclophosphamide significantly increased TA activity also, though the dose was smaller relative to LD_{50}. Adrenalectomy decreased the drug effects in almost all cases. Equi-toxic i.p. or p.o. doses of acetylsalicylic acid were equi-effective in intact rats. The action was dose-dependent and could be observed at a therapeutic dose. Elevated TA activities were determined up to 28 hrs after the last drug administration. Concomitant administration of glucose did not suppress the effect of mefenamic acid. The findings led to the following hypothesis: TA stimulation by NSAIDs depends on the actions of endogenous corticosteroids and, possibly, cyclic 3′,5′-AMP. Similar processes might play a role in the anti-inflammatory mechanism of NSAIDs.

In previous investigations we failed to demonstrate major stimulating effects of NSAIDs on microsomal drug-metabolizing enzymes and tryptophan pyrrolase in rat liver [1, 2]. Flufenamic acid, however, caused a marked increase in hepatic TA activity (EC 2.6.1.5) [2]. Both tryptophan pyrrolase and TA are glucocorticoid-inducible enzymes, underlying different physiological control mechanisms [3]; several stimuli affecting TA activity fail to influence tryptophan pyrrolase [3–8]. The observed difference in the response to flufenamic acid suggested that there might be differences also in the response of the enzymes to other NSAIDs.

In the past, several NSAIDs were found to inhibit aminotransferases *in vitro* [9–15], and it was concluded that these drugs might disturb amino acid metabolism *in vivo* [14]. Very recently, it was reported that salicylate increases TA activity *in vivo* [16].

It will be shown that TA was inhibited by several NSAIDs *in vitro*, but enhanced by the same and other NSAIDs *in vivo*. This stimulatory effect was dependent on adrenal glands for the main part, but it was apparently not restricted to glucocorticoid actions.

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EXPERIMENTAL

Materials. L-Tyrosine and pyridoxal-5-phosphate (Ferak Berlin), α-ketoglutaric acid (Reanal Budapest), diethyldithiocarbamate-sodium (VEB Laborchemie Apolda), methylcellulose (Tylose®, DHZ Laborchemie Berlin), dimethylsulfoxide (VEB Jenapharm Jena), gold sodium thiosulphate (Sanochrysin®, Ferrosan Kopenhagen) and ibuprofen (Boots Co., Nottingham) were used; the origin of the other drugs was specified previously [2].

Animals and pretreatment. The experiments were performed on colony-bred male Wistar rats (Uje: WIST) aged 3–4 months on average (for exceptions see table and figure legends). Housing conditions and diet were as described earlier [2]. Adrenalectomized animals were maintained on a regular diet and 1% NaCl solution instead of tap water until experimental use 4–5 days postoperatively. The mode of drug pretreatment was detailed previously; except for phenylbutazone, gold sodium thiosulphate, cyclophosphamide and chloroquine, single doses amounted to 20 per cent of

the p.o. LD_{50} [2]. Drug doses specified in the tables were given p.o. 28, 16 and 4 hr before decapitation, unless stated otherwise in the legends. This scheme was used according to Houck *et al.* [17], who gave drugs 28 and 4 hr before sacrificing; the administration at 16 hr was inserted in order to obtain more permanent drug levels in blood. In adrenalectomized rats, few drug doses were lowered; gold sodium thiosulfate and ibuprofen were studied only in intact rats. Vehicle volume was 10 ml/kg.

Enzyme source and assay. Four hr after the last drug administration, the animals were killed by decapitation at 2:00 to 3:00 p.m. After bleeding, livers were removed quickly and aliquots of 1 g fresh liver stored for about 45 min at 0° in 1·15% KCl solution. Liver pieces were then homogenized in a Potter–Elvehjem glass homogenizer with teflon pestle, yielding a 5% homogenate in 1·15% KCl (w/v). After centrifugation for 20 min at 85,000 *g*, the lipid layer was removed and the clear supernatant diluted 1:9 with 1·15% KCl. The diluted fraction was used as enzyme source. TA was assayed according to Wurtman and Axelrod [18] with minor modifications. Thus, all volumes were doubled, and 0·5 μmole diethyldithiocarbamate-sodium was added to each incubation tube. The enzymic reaction was started by addition of α-ketoglutarate. Protein was determined with the biuret method using bovine serum albumin as standard. To estimate the influence of drugs on TA *in vitro*, livers of untreated rats were prepared as described above. Pooled supernatants of three livers were used for enzyme assay. Drugs were added to the incubation mixture giving final concentrations of 10^{-5} to 10^{-3} M. Enzyme inhibition was calculated as per cent decrease of control activity. The type of inhibition was determined graphically according to Lineweaver–Burk [19], K_i values according to Dixon [20]. Benzydamine could not be studied because of its poor water solubility in the presence of concentrated KOH, added to stop the enzymic reaction.

RESULTS

Effects of NSAIDs on hepatic TA activity in vitro. As shown in Table 1, indomethacin, flufenamic acid, mefenamic acid, gold sodium thiosulphate and ibuprofen proved to be competitive inhibitors of TA *in vitro*. Kinetic analysis was carried out only when the agent in question showed clear inhibitory activity at 10^{-3} M. It is possible, therefore, that several of the drugs studied are inhibitors of TA at concentrations exceeding 10^{-3} M.

Effects of NSAIDs on TA activity in vivo. As shown in Table 2, all drugs but salicylamide significantly increased hepatic TA activity in normal animals. TA activity was enhanced 1·3- to 17·8-fold. The order of drug potency, referred to an equi-toxic dosage, was: mefenamic acid (17·8), ibuprofen (7·2), dimethylsulfoxide (6·8), flufenamic acid (5·5), acetylsalicylic acid (4·9), sodium salicylate (2·3), benzydamine (2·2), aminopyrine (2·1), gold sodium thiosulphate (2·1), and indomethacin (1·6). Also phenylbutazone (1·8), cyclophosphamide (1·4) and chloroquine (1·4) significantly increased TA activity, despite of their lower dosage relative to p.o. LD_{50}.

Adrenalectomy widely reduced this enhancing effect in almost all cases, except for indomethacin (2·4 vs 1·6), cyclophosphamide (1·8 vs 1·4) and aminopyrine (2·1 vs 2·0). Also acetylsalicylic acid (2·4), benzydamine (1·4) and dimethylsulfoxide (1·9) significantly increased TA activity in adrenalectomized rats.

In normal animals, NSAIDs did not reduce the effect of hydrocortisone. Potentiation of hydrocortisone by NSAIDs occurred with salicylamide, flufenamic acid, gold sodium thiosulphate, cyclophosphamide and ibuprofen; the effects were simply additive for phenylbutazone and mefenamic acid; in all other cases, the effects were partly additive.

In adrenalectomized animals, potentiation of hydrocortisone by NSAIDs occurred with acetylsalicylic acid, sodium salicylate, flufenamic and mefenamic acids. Benzydamine and phenylbutazone inhibited the

Table 1. Influence of NSAIDs on hepatic TA activity *in vitro*

Drug	% Inhibition at 1·0 mM	Apparent K_i (mM)	Type of inhibition relative to tyrosine
Acetylsalicylic acid	11		
Sodium salicylate	4		
Salicylamide	6		
Amidopyrine	4		
Phenylbutazone	5		
Indomethacin	62	0·19	competitive
Flufenamic acid	62	0·20	competitive
Mefenamic acid	84	0·15	competitive
Dimethylsulfoxide	7		
Gold sodium thiosulphate	93	0·07	competitive
Cyclophosphamide	0		
Chloroquine	7		
Ibuprofen	21	1·18	competitive

Table 2. Influence of NSAIDs and/or hydrocortisone on hepatic TA activity in intact and adrenalectomized rats

Drug	State of rats	Single dose (mg/kg)	TA activity: Control (1% Tylose®)	Hydrocortisone (5 mg/kg)	Drug alone	Drug plus hydrocortisone
Indomethacin	I‡	3	100·0 ± 11·8 (2·96)	326·0 ± 64·8*	157·5 ± 11·8*	269·0 ± 26·4
	A	3	100·0 ± 11·6 (2·33)	699·0 ± 27·8*	244·0 ± 26·2*	788·0 ± 64·0
Acetylsalicylic acid	I‡	300	100·0 ± 11·8 (2·96)	326·0 ± 64·8*	486·0 ± 58·1*	513·0 ± 58·1
	A	300	100·0 ± 10·9 (4·33)	445·0 ± 30·0*	236·0 ± 14·8*	658·0 ± 27·6†
Sodium salicylate	I	320	100·0 ± 8·1 (6·69)	234·0 ± 12·8*	228·0 ± 40·6*	325·0 ± 40·5
	A	120	100·0 ± 10·9 (4·33)	445·0 ± 30·0*	147·0 ± 20·3	569·0 ± 41·1
Salicylamide	I	400	100·0 ± 6·7 (4·81)	208·0 ± 38·3*	127·0 ± 10·4	372·0 ± 34·5†
	A	160	100·0 ± 11·6 (2·33)	699·0 ± 27·8*	90·5 ± 12·4	570·0 ± 75·2
Aminopyrine	I	340	100·0 ± 8·1 (6·69)	234·0 ± 12·9*	210·0 ± 24·5*	237·0 ± 36·8
	A	340	100·0 ± 0·8 (2·99)	579·0 ± 18·1*	198·0 ± 19·0*	624·0 ± 38·5
Benzydamine	I	200	100·0 ± 12·2 (4·82)	202·0 ± 27·6*	216·0 ± 34·4*	299·0 ± 36·3
	A	100	100·0 ± 4·5 (3·15) [6]	428·0 ± 10·8 [7]*	137·0 ± 16·2 [7]*	305·0 ± 20·6 [7]†
Flufenamic acid	I	90	100·0 ± 26·1 (2·07)	342·0 ± 26·1*	545·0 ± 120·0*	985·0 ± 119·0†
	A	90	100·0 ± 4·5 (3·15) [6]	428·0 ± 10·8 [7]*	119·0 ± 16·2 [3]	963·0 ± 39·6 [6]†
Mefenamic acid	I	340	100·0 ± 11·7 (1·54)	520·0 ± 22·2*	1780·0 ± 94·3*	2320·0 ± 51·4†
	A	340	100·0 ± 4·9 (3·27)	392·0 ± 25·4*	77·8 ± 5·5	449·0 ± 29·9
Dimethylsulf-oxide	I	3500	100·0 ± 11·7 (1·54)	520·0 ± 22·1*	682·0 ± 38·3*	1005·0 ± 36·4†
	A	3500	100·0 ± 6·6 (3·16)	606·0 ± 41·5*	192·5 ± 12·7*	645·0 ± 32·3
Phenylbuta-zone	I	50	100·0 ± 6·5 (1·68)	405·0 ± 17·3*	188·0 ± 10·1*	497·0 ± 43·5
	A	50	100·0 ± 3·4 (3·20)	513·0 ± 21·3*	79·8 ± 4·4	345·0 ± 30·0†
Chloroquine diphosphate	I	20	100·0 ± 6·0 (2·98)	295·0 ± 7·4*	139·0 ± 10·2*	264·0 ± 17·4
	A	20	100·0 ± 3·6 (3·20)	513·0 ± 20·0*	112·8 ± 6·3	468·0 ± 11·2
Cyclophospha-mide	I	30	100·0 ± 6·0 (2·98)	295·0 ± 7·4*	139·0 ± 3·7*	408·0 ± 9·7†
	A	30	100·0 ± 6·6 (3·16)	606·0 ± 41·4*	179·0 ± 4·1*	484·0 ± 23·4
Gold sodium thiosulphate	I	30§	100·0 ± 15·0 (1·73)	549·0 ± 12·7*	206·0 ± 38·8*	838·0 ± 63·6†
Ibuprofen	I	320	100·0 ± 6·9 (4·81)	208·0 ± 38·3*	720·0 ± 35·2*	1160·0 ± 76·9†

Means ± S.E.M. Results were normalized to per cent of control activity; original values of controls are given in parenthesis as μmole *p*-hydroxyphenylpyruvate × [10 mg protein]$^{-1}$ × [20 min]$^{-1}$. The number of intact animals was 6, that of adrenalectomized rats 5 per group, unless specified otherwise by numbers in brackets.

* Significantly different from controls (*t*-test; $P \leq 0{\cdot}05$).

† Significantly different from all foregoing values in the same line at $P \leq 0{\cdot}05$.

‡ Rats used in this experiment were 8 months-old.

§ Gold sodium thiosulphate was injected i.m., dissolved in 0·5 ml saline per 100 g body wt. TA assay was performed without adding diethyldithiocarbamate sodium.

I = intact; A = adrenalectomized.

effect of the hormone, while in the other cases, the effects were partly additive.

Control TA activities were the same in normal and adrenalectomized animals. TA activity was more enhanced in adrenalectomized than in normal rats by hydrocortisone.

There was a rough parallelism between degree of injury of the gastrointestinal tract and TA stimulation in intact animals: fenamates, ibuprofen, salicylates (except for salicylamide), benzydamine and aminopyrine caused disturbances, ranging from ulcers and erosions of the mucous membrane of the stomach to peritoneal exudation, adhesions, or simply maximal inflation of the stomach. In fenamate- and acetylsalicylic acid-treated animals, macroscopic gastrointestinal bleeding was observed. Aminopyrine, fenamates and benzydamine caused deaths in adrenalectomized animals. Benzydamine dosage had to be lowered therefore, since mortality rate exceeded 50 per cent. Simultaneous treatment with hydrocortisone reduced toxicity; the condition of those animals was better than after nonsteroids alone.

Influence of the route of drug administration. As the comparison of Tables 3 and 2 shows, acetylsalicylic acid apparently increased TA activity more distinctly after i.p. than after p.o. administration. However, the 1% Tylose® solution used as drug vehicle contributed to the stimulation of TA, causing a 3-fold increase in activity itself, as compared with saline-treated controls. Thus, when the vehicle effect was subtracted, acetylsalicylic acid proved to be equi-effective at equi-toxic doses, irrespective of the route of administration.

Time-dependence of TA stimulation by acetylsalicylic acid. As shown in Fig. 1, TA activity reached maximal values as soon as 5 hr after the first administration of acetylsalicylic acid; then it remained at its elevated level up to 6 hr following the last drug administration; thereafter, a slow decrease was observed. TA activity had not yet reached control values even 28 hr after the last dosage.

Table 3. Influence of acetylsalicylic acid and/or hydrocortisone on hepatic TA activity in intact rats

Pretreatment and route of administration	No. of rats	TA activity [μmoles p-HPP × (10 mg protein)$^{-1}$ × (20 min)$^{-1}$]
Saline i.p. (10 ml/kg)	6‡	4·03 ± 0·52
1% Tylose i.p. (10 ml/kg)	6‡	12·82 ± 1·73*
Acetylsalicylic acid (100 mg/kg i.p.), suspended in 1% Tylose	5‡	26·17 ± 4·45†
None (no handling)	12	2·80 ± 0·25†
1% Tylose p.o. (10 ml/kg)	12	4·50 ± 0·60

Means ± S.E.M. Drug dosage was 20% of the acute i.p. LD_{50}, three times.
* Significantly different from saline-treated controls at $P \leq 0{\cdot}05$, t-test.
† Significantly different from tylose-treated controls (i.p. vs i.p., p.o. vs "no handling") at $P \leq 0{\cdot}05$; t-test.
‡ Rats used in this experiment were 60 days-old.
p-HPP = p-Hydroxyphenylpyruvate.

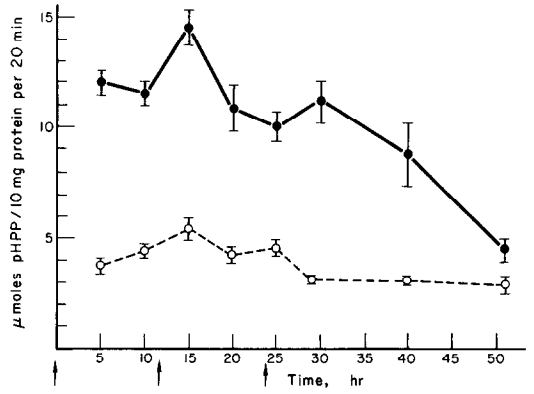

Fig. 1. Time-dependence of the TA-stimulating effect of acetylsalicylic acid. Means ± S.E.M. Intact rats aged 8 months were used. Arrows indicate p.o. administrations of 300 mg/kg acetylsalicylic acid. (●) Drug-treated animals. (○) Vehicle-treated controls. All values of the upper curve are significantly different from controls at $P \leq 0{\cdot}05$; t-test. For statistics see Table 2.

Dose–response relationship of TA stimulation by acetylsalicylic acid. As drug doses used in our experiments exceeded therapeutic levels, we wondered, whether TA stimulation was demonstrable also at lower dosage. As shown in Table 4, a significant increase in TA was found at single doses of 50 mg/kg, i.e. 100 mg/kg per 24 hr, a therapeutic dosage.

Failure of glucose to repress TA stimulation by mefenamic acid. Starvation results in TA induction, and glucose administration is able to repress this increase in TA activity [21, 22]. Pretreatment with drugs often caused inappetence of the rats in our investigations. Therefore, mefenamic acid was administered together with a large amount of glucose. As shown in Table 5, glucose failed to repress the drug-induced TA increase. Evidently, the mefenamic acid effect is not due to a glucose deficiency.

Table 4. Dose–response relationship of the TA-stimulating effect of acetylsalicylic acid given p.o.

Acetylsalicylic acid dose	TA activity [μmoles p-HPP × (10 mg protein)$^{-1}$ × (20 min)$^{-1}$]
Control (1% Tylose)	3·26 ± 0·38
50 mg/kg	5·82 ± 0·66*
100 mg/kg	7·94 ± 0·92*
200 mg/kg	10·29 ± 1·10*
300 mg/kg	9·31 ± 0·64*

Rats were 60 days-old.
Symbols for statistic differences as in Table 2.

DISCUSSION

Drug effects in vitro. Inhibition of aminotransferases by NSAIDs was observed by several investigators [10–15]. Whitehouse discussed the possible meaning of these changes in amino acid metabolism [9, 23].

In our experiments, indomethacin, gold sodium thiosulphate, flufenamic acid, mefenamic acid and ibuprofen proved to be inhibitors of TA *in vitro*. Salicylates, pyrazole derivatives, dimethylsulfoxide and two immunosuppressants did not influence TA activity at concentrations lower than 10^{-3} M. Other workers, who were able to demonstrate inhibition of aminotransferases by salicylate [15] or phenylbutazone [10] *in vitro*, used higher drug concentrations.

NSAIDs including indomethacin, fenamates and gold salts are inhibitors of histidine decarboxylase [24], probably by competition with the co-factor pyridoxal-5-phosphate for lysyl epsilon-amino groups, the presumable binding site for co-enzyme [23]. 5-Hydroxytryptophan decarboxylase, another pyridoxal-5-phosphate-dependent enzyme, is also inhibited by NSAIDs [25]. In this case, however, drugs compete with the substrate, rather than with co-enzyme, for receptor sites. As we could demonstrate, TA inhibition by NSAIDs also is due to competition with substrate.

Table 5. Influence of mefenamic acid and/or glucose on hepatic TA activity in intact rats

Pretreatment	Dose	Number of animals	TA activity [μmoles p-HPP $\times$ (10 mg protein)$^{-1}$ $\times$ (20 min)$^{-1}$]
Control (1% Tylose®)	10 ml/kg	6	4·90 ± 0·63
Glucose	6·0 g/kg	6	3·70 ± 1·04
Mefenamic acid	340 mg/kg	5†	35·05 ± 11·07*
Mefenamic acid + glucose	340 mg/kg + 6·0 g/kg	5†	43·60 ± 14·50*

8 months-old rats were used.
Symbols for statistic differences as in Table 2.
† One animal died during treatment.

In contrast to 5-hydroxytryptophan decarboxylase [25], TA is inhibited by gold sodium thiosulphate.

Drug effects in vivo. In contrast to drug effects *in vitro*, all NSAIDs (except for salicylamide) increased hepatic TA activity in intact rats. Cytosol was diluted at least 2000-fold before TA assay, so that drug levels present in the livers at the time of killing may be neglected. The comparison of the increase of TA activity *in vivo* and its inhibition by several drugs *in vitro* suggests that the inhibitory influences were compensated or even overcompensated by affecting the complex control mechanisms of TA [3, 26–28] *in vivo*. There was no relation between TA inhibition by one compound *in vitro* and its stimulatory effect *in vivo*.

The discussion, whether NSAIDs act directly or via glucocorticoids, was reviewed by Domenjoz [29], and, specially for salicylates, by Smith and Smith [30]. As demonstrated in our experiments on adrenalectomized rats, adrenal glands played an essential role in the TA-increasing action of NSAIDs; probably, glucocorticoids are the responsible adrenal factors, though one might take into account catecholamines as well [31, 32]. TA is a very sensitive indicator of many kinds of stress, like starvation, infection, poisoning etc., considered to lead to an activation of the hypophyseo-adrenal system [5, 7, 8, 21, 33–39]. Also the increase of TA activity following i.p. injection of methylcellulose in our experiments lies on this line.

However, some of the findings are hardly consistent with the assumption of a merely glucocorticoid-mediated increase of TA activity by NSAIDs. Several drugs increased TA activity also in adrenalectomized animals. In intact rats, acetylsalicylic acid increased enzyme activity at a therapeutic dosage, when stress ought to be minimal. In a recent paper, we reported the failure of NSAIDs to affect hepatic tryptophan pyrrolase, whereas hydrocortisone reliably induced this enzyme [2]; doses and treatment pattern were the same as in the present investigations. This discrepancy led us to the following hypothesis. Stimulation of hepatic TA by NSAIDs in intact rats results from the complex action of both adrenal factors (supposedly glucocorticoids) and extra-adrenal factors. Apparently, a mechanism of synergistic enhancement is working [40]. The discrepancy mentioned above resolves, when cyclic 3',5'-AMP (cAMP) is assumed to be the responsible extra-adrenal factor. cAMP induces TA, but not tryptophan pyrrolase, and enhances induction of TA by hydrocortisone synergistically [5, 6, 41]. It is not clear, how NSAIDs might trigger the synthesis of cAMP. Possibly, glucagon is an intermediate, for glucagon also fails to induce tryptophan pyrrolase, but enhances TA induction by hydrocortisone [5, 41]. Our earlier assumption that a drug-conditioned glucose deficit, which is known for salicylate for instance [30], might be the trigger for the TA increase, could not be confirmed. Perhaps, factors other than glucocorticoids and glucagon are involved, too [16].

TA increase seems to be connected with drug-induced lesions of the digestive tract. The severity of the drug-induced gastrointestinal injury was related to the enhancement of TA activity in the liver. It cannot be decided from the present study whether the TA increases due to NSAIDs were consequences of, or parallel events with, injury of the gastrointestinal tract. The finding that TA activity reached maximal values as soon as 5 hr after p.o. administration of acetylsalicylic acid would indicate a parallel or even preceding, rather than ensuing, effect on TA. Thus, it seems possible that gastrointestinal lesions and TA increase are different drug effects, depending on the same or closely related mechanisms. In this connection, the findings of Bhargava *et al.* [42] are interesting. They reported that the ulcerogenic action of NSAIDs is due, at least in part, to a central activation of the sympathetic system. Pretreatment with alpha-sympatholytics clearly reduced the frequency of gastrointestinal lesions [42]. Analogous findings were reported recently for aspirin [43]. These observations are important with respect to the assumed mechanism of TA stimulation by NSAIDs, since catecholamines are known to enhance both cAMP formation and TA activity [32]. In this context, catecholamines also deserve consideration as important adrenal factors, possibly together with glucocorticoids (cf. above). We were unable, however, to prevent or diminish the increase of TA due to acetylsalicylic acid by ganglionic blockade with hexamethonium.*

* Unpublished own results.

Though the exact mechanism of TA stimulation by NSAIDs remains to be elucidated, our investigations might contribute to an understanding of the mode of action of this group of drugs. Provided that processes of enzyme induction were involved in the anti-inflammatory action, mechanisms as discussed above could be the basis for a supposed, but till now unproved indirect action of NSAIDs. As already discussed by M. J. H. Smith in 1966 in his monograph "The Salicylates", this indirect action might consist of a reinforcement of effects of endogenous steroids [30] in body tissues.

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