out that the very large induction of DMN-demethylase by fasting and by feeding a carbohydrate-devoid protein diet was first reported from our laboratory [3]. In describing their own finding with fasting, which confirms our result, McLean and Day [15] failed to note the priority of our report [3], yet it was cited by them in another context.

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Seamen's Memorial Research Laboratory, U.S. Public Health Service Hospital, 210 State Street, New Orleans, La. 70118, and Department of Medicine, Tulane University Medical Center, New Orleans, La, and The Salk Institute La Jolla, Calif., U.S.A.

JOSEPH C. ARCOS*
GEORGIA M. BRYANT
NATARAJAN VENKATESAN
MARY F. ARGUS

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* Send reprint request to the first author at the first address.

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Effect of ATP upon dopamine-β-hydroxylase

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A stimulating effect of ATP upon the conversion of dopamine to noradrenaline was first reported when crude adrenal extracts were used [1, 2]. This effect, however, was much less apparent when the dopamine-β-hydroxylase (DBH) was purified; in this case the ATP caused only a 1·5-fold stimulation instead of the 5–10-fold stimulation noted when crude adrenal extracts were used [3]. As even this slight effect disappeared when initial rates of DBH were measured, it was concluded that ATP was not intimately involved in the hydroxylation reaction [4]. The fortuitous observation that a nucleotide preparation from a different origin was ineffective, even with a crude extract, prompted us to investigate a possible interaction between the apparent stimulation of DBH by ATP and the presence of endogenous inhibitors.

Fresh bovine adrenal glands were obtained from the local slaughterhouse and immediately chilled in ice-cold 0·25 M sucrose. The medullae were dissected from the total gland, minced and then homogenized in 10 vol 0·25 M sucrose with a Duall homogenizer. The total homogenate was centrifuged at low speed (1000 *g* for 10 min) and the pellet was discarded. The supernatant was further centrifuged at 27,000 *g* for 10 min and the pellet, corresponding to the M + L fraction described in a previous paper [5], was washed twice and resuspended in 20 vol. The final supernatant fraction was obtained by centrifugation at 120,000 *g* for 60 min.

Another fractionation procedure—that described by Kuzuya and Nagatsu [6]—was also followed. In this case the medullae were homogenized with an Ultraturrax in 6 vol of a 0·25 M sucrose solution containing 0·02 M potassium phosphate buffer (pH 7·0). After a low speed run, a particulate fraction was obtained by centrifugation at 20,000 *g* for 30 min.

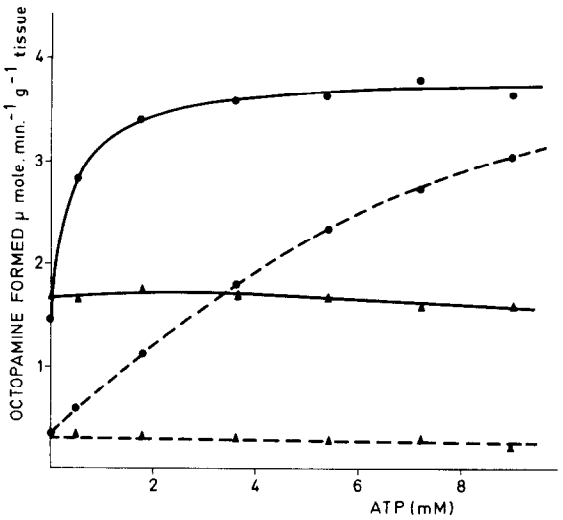

Fig. 1. DBH activity in a M + L fraction (dilution 1:20) from bovine adrenal medullae as a function of ATP concn in the presence (——) and absence (– – – –) of *N*-ethylmaleimide (3×10^{-2} M). (●) ATP from Sigma; (▲) ATP from Boehringer.

DBH was measured by means of a spectrometric method using tyramine as substrate. The incubation conditions have been already described in detail [7]. The structure-linked latency of DBH was estimated biochemically as the difference between the activity assayed under isotonic conditions—which tend to respect the integrity of the particles (free activity)—and the activity determined in the presence of Triton X-100, which disrupts the particles (total activity). Two different preparations of ATP were used: one supplied by Sigma (adenosine 5′ triphosphate di-sodium salt from equine muscle) and one from Boehringer (adenosine 5′ triphosphate di-sodium salt 15028).

The total DBH activity was measured from an M + L fraction by increasing the ATP concentration in the presence and in the absence of *N*-ethylmaleimide. Figure 1 shows that the ATP from Boehringer had no effect at all on DBH activity in either experimental condition. In contrast to this, the Sigma ATP was found to stimulate DBH activity tremendously, particularly when *N*-ethylmaleimide was omitted. Moreover, even in the presence of the latter, a clearcut stimulating effect was observed at low concentrations of the ATP. These results therefore suggest that ATP might have the same stimulating effect (and perhaps even more) as *N*-ethylmaleimide by reversing the effects of DBH endogenous inhibitors. This hypothesis was confirmed by measuring the copper content [8] of both nucleotide preparations: ATP from Sigma contained 0·2 μg Cu^{2+}/mg while Boehringer ATP contained less than 0·01 μg/mg. Consequently, the stimulation of DBH activity by ATP was not due to the nucleotide itself but rather to copper contamination of certain ATP preparations. It is well-known that copper, just like *N*-ethylmaleimide, can reverse the effects of endogenous inhibitors of DBH [9–11].

Table 1 shows the effect of ATP, *N*-ethylmaleimide and Triton X-100, at various concentrations, on three different subcellular fractions. The M + L fraction was found to exhibit the lowest DBH free activity, and therefore had up to 90% of latency.

It was noteworthy that, in the presence of *N*-ethylmaleimide, the free activity as a percentage of total activity was somewhat higher, thus confirming the results of our previous studies [11]. The total DBH activity in this fraction was practically not increased by *N*-ethylmaleimide, as compared with the activity obtained with ATP. In the supernatant fraction, however, DBH activity became 5 times higher after the addition of *N*-ethylmaleimide than after the addition of ATP only. This suggests that the supernatant contains far more endogenous inhibitors than the M + L fraction, thus confirming our earlier findings [11]. Nevertheless, in the absence of Sigma ATP (i.e. in the absence of copper), the total DBH activity in the M + L fraction was much less than with ATP—a clear indication of the presence of endogenous inhibitors. In our previous studies [11] this effect was not observed as we used Sigma ATP for all the DBH assays. The particulate fraction, prepared according to Kuzuya and Nagatsu, [6] was found to exhibit a high DBH free activity, which suggests that the granules suffer more damage in the Ultraturrax homogenizer than in the Duall. This preparation also contained far more endogenous inhibitors than the M + L fraction since DBH activity was further enhanced by the addition of *N*-ethylmaleimide. Since the endogenous inhibitors are sulfhydryl compounds and since many proteins contain SH-groups it seems logical to suppose that such inhibition could occur virtually everywhere. However, our results indicate the inhibitors are present in much larger amount in the supernatant, while the DBH activity is highest in the M + L fraction. Under our experimental conditions, Triton X-100 at a concentration of 0·2% did not

Table 1. Effect of Triton X-100, ATP and NEM on dopamine-β-hydroxylase activity in different fractions of bovine adrenal medulla

Fraction	Addition: NEM 3×10^{-2} M	Addition: ATP 9×10^{-3} M	Dopamine-β-hydroxylase activity (μmoles/min per g tissue): Triton X-100 (%) None	0·01	0·05	0·2	Free activity (% of total)*
M + L	–	+	0·42	1·67	4·11	4·00	10
(dilution 1:20)	–	–	0·01	0·082	0·34	0·14	7
	+	+	0·75	2·56	4·75	4·81	16
	+	–	0·37	1·17	2·25	1·76	21
Supernatant	–	+	0·44	0·37	0·23	0·29	—
(dilution 1:20)	–	–	0·06	0·07	0·05	0·05	—
	+	+	1·64	1·53	1·48	1·52	—
	+	–	0·47	0·43	0·33	0·36	—
Particulate	–	+	1·21	2·90	3·35	3·18	38
(dilution 1:20)	–	–	0·17	0·25	0·39	0·34	50
	+	+	2·49	4·55	6·26	6·14	40
	+	–	1·71	2·56	3·77	3·35	51

* Total activity represents the activity measured with 0·2% Triton.

significantly inhibit DBH activity, in contrast to the results of Kuzuya and Nagatsu [6]. Of interest here is the fact that 0·05% Triton X-100 is still sufficient to measure the total DBH activity.

In conclusion, it appears that ATP in itself exerts no direct stimulation on DBH. However some ATP preparations which are contaminated by copper may artificially increase DBH activity by reversing the effects of endogenous inhibitors. As DBH activity is generally measured with or without ATP the present results provide a good explanation for the considerable discrepancies which exist between numerous reports as regards the copper concentration required to produce maximal DBH activity.

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Department of Neurobiochemistry,
Janssen Pharmaceutica,
B-2340 Beerse,
Belgium

PIERRE M. LADURON

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Influence of adrenocorticotropic hormone, somatotrophic hormone and pregnenolone-16α-carbonitrile on drug response and metabolism

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In intact unlike in adrenalectomized rats, pretreatment with adrenocorticotropic hormone (ACTH) reduces the effects of certain drugs [1]. This action can be only partially simulated by corticosterone, suggesting that some steroids are involved in the effect of ACTH upon drug responses [1].

Somatotrophic or growth hormone (STH) is another agent that influences drug responses [2, 3]. Given as a pretreatment with ACTH, it abolishes the latter's protective effect upon acrylonitrile-induced adrenal apoplexy and mortality [4]. In rats, conjoint treatment with STH and pregnenolone-16α-carbonitrile (PCN) counteracts the beneficial action of the steroid against digitoxin or indomethacin intoxication [5, 6].

PCN, a synthetic nonhormonal catatoxic steroid, possesses no other known effect besides its ability to reduce the toxicity of a large number of drugs [2]. In most cases, its actions are mediated through the induction of drug-metabolizing enzymes in hepatic microsomes [7–10], but it also stimulates certain extramicrosomal enzymes, for example, phosphoprotein phosphatase [11]. Recently, it was found that the reduction of zoxazolamine paralysis time by PCN, unlike that caused by ACTH, is associated with decreased drug concentrations in plasma [12]. This protective effect of ACTH is shared by triamcinolone and corticosterone in that the diminution of zoxazolamine paralysis is not accompanied by lower plasma levels of the toxicant [12, 13]. Distribution studies [14, 15] have not explained the reduction of zoxazolamine paralysis by ACTH and corticoids. Hence, it seemed of value to compare the effects of these agents on drug metabolism. Preliminary reports on this subject have appeared elsewhere [16, 17].

Female Charles River CD® rats (Canadian Breeding Farms & Laboratories Ltd., St. Constant, Que.), with an initial average body weight of 100 g, were maintained *ad lib.* on Purina Laboratory Chow (J. Mondu Inc., Montreal, Que.) and tap water. Unless otherwise stated, each group consisted of 10–15 rats. Every experiment was repeated two to three times.

In the first experiment, PCN (3β-hydroxy-20-oxo-5-pregnen-16α-carbonitrile (Upjohn) was given p.o. (by stomach tube) at a dose level of 1 mg in 1 ml water (as a microcrystal suspension after addition of a trace of polysorbate or Tween 80) twice daily on the first, second and third day and once on the fourth day, 1 hr before zoxazolamine (K. & K. Laboratories) in 1 ml distilled water (homogenized with a trace of polysorbate or Tween 80). The wet adrenal weights were determined on day 5.

In the second experiment, 5 I.U. of depot ACTH was injected s.c. 24 hr before zoxazolamine (given as described in the first experiment). Bovine STH (Upjohn) was administered once s.c., at a dose level of 2 mg or 5 U (in 0·2 ml distilled water) 28 and 4 hr before the zoxazolamine injection.

In the third experiment, 0·1 mg PCN (a dose just sufficient to diminish zoxazolamine paralysis) was given p.o. twice daily on days 1, 2 and 3 and once on the day 4, 1 hr before zoxazolamine (administered as in the first experiment). STH was injected s.c., always 1 hr before PCN, in doses that were identical to those used in the second experiment.

In the fourth experiment, 1 mg PCN was given twice daily p.o. for 3 days. Depot ACTH (5 I.U.) was injected once s.c. 24 hr before the rats were decapitated (on day 4, approximately 17 hr after the last PCN gavage).

The livers were immediately excised, weighed and washed in an ice-cold aqueous solution of 1·15% KCl, containing 0·02 M tromethamine Tris–HCl buffer, pH 7·4. All the hepatic tissues were subsequently processed at 0–4°,