when sensitized mast cells were incubated with WGA bound to Sepharose 6 B beads, some 30 per cent of the cells showed a generalized pattern of degranulation in the presence *or* absence of calcium.

In summary, we have found that the lectin wheat germ agglutinin (WGA) stimulates granule extrusion and histamine release from isolated rat mast cells. This secretory response occurs in the absence of extracellular calcium but is prevented by pretreating the mast cells with a chelating agent and restored by the reintroduction of calcium (but not magnesium) to the bathing medium. This secretory response to WGA is also prevented by energy deprivation or by the sugars, NANA or NAG, to which WGA specifically binds. Unlike the lectin, Con A, WGA is able to elicit this secretory response from non-sensitized mast cells in the absence of the cofactor phosphatidyl serine. It is suggested that WGA initiates secretion by mobilizing a "cellular" or bound source of calcium and thus resembles the mast cell secretagogue, 48/80 [27].

Acknowledgements—We thank the Tufts Faculty Research fund for support to D.E.C. A23187 was a gift from Eli Lilly Research Laboratories.

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Biochemical Pharmacology, Vol. 29, pp. 458–460 0006-2952/80/0201-0458 $02.00/0

Induction of δ-aminolevulinic acid synthetase in chick embryo kidney

(*Received* 27 *March* 1979; *accepted* 21 *August* 1979)

δ-Aminolevulinic acid (ALA) synthetase is the rate-limiting enzyme of porphyrin and heme synthesis. Induction of this enzyme by porphyrinogenic agents is most marked in the liver. The response of this organ to various chemicals is the basis of *in vivo* or *in vitro* systems for assessing the induction potential of these agents [1–3]. Heme synthesis and turnover in kidney have rates and magnitudes comparable to those of liver in some species [4]. However, ALA synthetase induction in kidney has not been studied extensively. Barnes *et al.* [5] described the induction of ALA synthetase activity in kidney mitochondria in rats treated with allylisopropylacetamide (AIA). Schwartz *et al.* [6] were unable to induce ALA synthetase in Syrian hamster kidney with 3,5-dicarbethoxy-1,4-dihydrocollidine (DDC). We previously reported significant induction of ALA synthetase in the kidneys of 1-day-old chicks treated

with AIA. DDC failed to induce ALA synthetase in chick kidney, although marked induction occurred in the liver in this system [7]. Using a 20-day-old chick embryo system, we have now been able to document DDC-mediated induction of ALA synthetase in kidney as well as in liver.

ALA synthetase assay methods were modified because of the higher levels of ALA dehydratase in embryonal liver and kidney homogenates. To block ALA dehydratase activity, levulinic acid was added and the concentration of EDTA was increased. These modifications of the method of Hayashi *et al.* [8] yield higher levels of ALA synthetase in both embryonic and newly hatched chicks.

Fertilized eggs of a strain of White Leghorns were obtained from the Poultry Department of the University of Manitoba. Drugs (6 mg DDC or 20 mg AIA) were dissolved in 0.1 ml dimethylsulfoxide (DMSO) and introduced through the chorioallantois of eggs containing 20-day-old chick embryos. At intervals, livers and kidneys were removed and frozen at −20°. Control embryos were given only DMSO. Homogenates of frozen and thawed organs were made in 0.05 M Tris–HCl, pH 7.6, containing 0.1mM pyridoxal phosphate, and ALA synthetase activity was measured in the homogenates. The assay mixture contained in a final volume of 1 ml: 50 mM Tris–HCl, pH 7.7, 200 mM glycine, 10 mM succinate, 10 mM ATP, 0.05 mM CoA, 0.1 mM pyridoxal phosphate, 6 mM EDTA, 6 mM $MgCl_2$, 7.5 mM levulinic acid, succinyl CoA synthetase generating 1.2 μmoles succinyl CoA/hr, and appropriate amounts of homogenate. The reaction was carried out in a test tube for 30 min at 37° in a shaking water bath and stopped by the addition of 0.25 ml of 12.5% trichloroacetic acid. The ALA formed was measured by a colorimetric method after condensation with acetylacetone [9]. Succinyl CoA synthetase was prepared from *Escherichia coli* [10]. Protein was measured by the biuret method [11], and the enzyme activity expressed as the generation of ALA per mg of protein per hr. AIA was a gift of Hoffmann-LaRoche Inc., Montreal, Canada. DDC was obtained from Eastman Organic Chemicals, Rochester, NY.

Although porphyrin and heme synthesis occurs in most eukaryotic cells, little is known about the basal levels and inducibility of the rate-limiting enzyme, ALA synthetase, in most eukaryotes. The induction of ALA synthetase by drugs has been documented primarily in liver cells. This may be due to selective affinity of the drug or chemical for that organ, to cell permeability, or to transformation of the drug to a porphyrinogenic metabolite in the liver. There are several reports of the inability of porphyrinogenic agents to induce ALA synthetase in organs other than liver. AIA does not induce ALA synthetase in heart, brain [5] or adrenals [12]. DDC also failed to induce the enzyme in adrenals [12], spleen [13] or heart [14]. However, hormones may induce in their natural target organs, such as progesterone inducing ALA synthetase in estradiol-primed chick oviduct [15], adrenocorticotropin in the adrenal [12], erythropoietin inducing ALA synthetase in mouse spleen [13], and certain 5β-reduced steroids in cultured chick blastoderm [16]. Higher ALA synthetase activity was found in this experimental system than in earlier experiments using newly hatched chicks [7] or embryonal liver cells [17]. This is, in part, related to the change in methodology in utilizing levulinic acid and EDTA to block ALA dehydratase. The greater induction may also be related to the higher sustained concentrations of the inducers in the closed embryo system.

We reported previously that ALA synthetase in kidney was not induced by DDC (6 mg) given as two injections 12 hr apart to newly hatched chicks. That the drug was active in the kidney was documented by the drop in kidney ferrochelatase activity. Marked induction of liver ALA synthetase (16-fold) was obtained [7]. In the present experiment, administration of the same dose of DDC to 20-day-old embryos induced ALA synthetase in both kidney and liver. The magnitude of induction was 4-fold in kidney and 12-fold in liver (Table 1). Another inducing agent, AIA, known to induce both liver and kidney ALA synthetase [7], caused a 15-fold induction in kidney and a 5-fold induction in liver in this chick embryo system, though the absolute levels of the enzyme attained in the kidney were less than in liver.

Table 1. Effects of DDC and AIA on chick embryo kidney and liver ALA synthetase activity*

	ALA synthetase† (nmoles ALA/mg protein/hr)	
	Kidney	Liver
Normal‡	1.5 ± 0.2 (8)	5.4 ± 0.8 (8)
Control‡	1.6 ± 0.2 (8)	7.6 ± 1.8 (8)
DDC	5.7 ± 0.5§ (6)	96.5 ± 16.3§ (6)
AIA	23.4 ± 2.7§(12)	38.5 ± 3.7§(12)

* DDC (6 mg) or AIA (20 mg) was dissolved in 0.1 ml DMSO and injected into the egg 18 hr prior to the removal of kidney and liver.

† Each value represents the mean ± S.E.M. The figures in parentheses represent the number of embryos.

‡ Normal denotes untreated embryos, and control denotes embryos injected with the vehicle only (DMSO).

§ Denotes significant difference from control values ($P < 0.001$) determined by Student's *t*-test.

These findings are in contrast to those obtained in the newly hatched chick which is chronologically only 1–2 days older [7]. In previous experiments, 1-day-old chick kidney ALA synthetase was not induced by DDC, whereas the degree of its induction by AIA in liver was greater than that in the kidney (the reverse of that in the embryo) [7]. The present assay method yields values for the levels of ALA synthetase in the kidney of 1-day-old chicks which are twice as great as values obtained with the previous method [7]. Also, using the present more sensitive modification of the assay for ALA synthetase, DDC was found to produce a small increase in ALA synthetase activity in 1-day-old chick kidney.

Differences in the perinatal period were observed by Song *et al.* [18] who found that ALA synthetase was refractory to induction by AIA in the perinatal rat and postulated that this enzyme belongs to a group whose appearance, activity and inducibility are developmentally determined. The species, sex and developmental differences in chicken liver ALA synthetase inducibility were also discussed by Creighton and Marks [19], who found that hepatic ALA synthetase activity was readily induced by drugs in the livers of newborn chickens.

The reason for the differences in inducibility of the 20-day-old chick embryo kidney and the 1-day-old chick kidney with DDC is not forthcoming from the present experiment. Developmental differences may exist in the embryos as compared with newly hatched chicks, or, higher concentrations of the inducing drugs may be attained in the chick embryo system where the excretion of drug is absent.

In summary, DDC has been demonstrated to induce ALA synthetase in kidney as well as liver when the 20-day-chick embryo is used as the test system. The magnitude of the induction of the enzyme per mg of organ protein by DDC was 4-fold in the kidney and 12-fold in the liver. By comparison, AIA produced a 15-fold induction in the kidney and a 5-fold induction in the liver as compared with controls. The characteristics of induction in this species are dependent on whether the embryo or the chick is used as the test system.

Acknowledgements—This work was supported by grants

from the Medical Research Council of Canada and the Mrs. James A. Richardson Foundation.

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Biochemical Pharmacology, Vol. 29, pp. 460–462
0006-2952/80/0201-0460 $02.00/0

Interaction of tricyclic antidepressants with opiate receptors

(*Received* 1 *August* 1979; *accepted* 7 *September* 1979)

Tricyclic antidepressants have been reported to have an analgesic effect [1,2] and some of them have been successfully used to alleviate chronic pain in man [3–6]. It is not, however, clear whether the analgetic effect of antidepressants exists in animals, due to the fact that different methods were used to measure pain [7,8], nor is the mechanism through which antidepressants exert their analgesic effects known [7]. We wish to report evidence for a direct

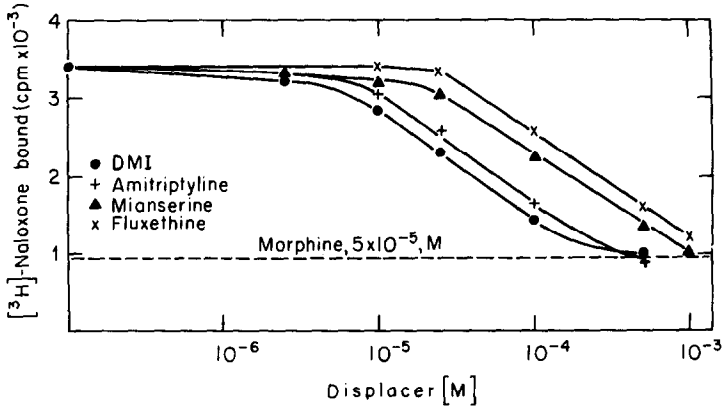

Fig. 1. Displacement of ^{3}H-naloxone binding by antidepressants. Female Wistar rats (3 months old) from the breeding colony of the Hormone Research Department of the Weizmann Institute of Science, were decapitated, their forebrains quickly removed, weighed and homogenized in 50 mM Tris–HCl Buffer, pH 7.7, in a polytron homogenizer. The homogenate was spun at 30,000 *g* for 10 min, the supernatant decanted and the pellet resuspended in buffer, homogenized and incubated for 45 min at 37°. Following a second centrifugation at 30,000 for 10 min, the pellet was resuspended in buffer and homogenized. A final volume of 200 ml per 3 of the original, weighed forebrains was used. Tissue suspension (2 ml) was added to test tubes containing 5 nM ^{3}H-naloxone (N.E.N. specific activity 15.2 Ci/mmole, 1 mCi/ml) together with (a) an unlabelled inhibitor (10^{-5}M naloxone or 5×10^{-5}M morphine); or (b) the various antidepressants or buffer to a final volume of 2.3 ml. After incubating for 45 min at room temperature (25°) the contents of each test tube were filtered through GF/B filters and washed 4 times in 5 ml ice cold buffer. The filter papers were shaken with a toluene triton scintillation mixture in vials and counted in a Packard Tri-Carb Scintillation Counter.