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## Inotropic Activity of a Series of Amino Acids.* (29270)

JOHN GATGOUNIS AND WILLIAM HESTER (Introduced by R. P. Walton)
*Department of Pharmacology and Therapeutics, Medical College of South Carolina, Charleston,*

The ability of the body to decarboxylate amino acids into more potent vasoactive amines has been indicated by previous studies(1,2,3). However, the initial reactants, that is, the amino acids, have generally been considered to be relatively inert circulatory agents(3). In this study a series of amino acids were examined in terms of their acute inotropic and pressor effects when administered in anesthetized, open-chest dogs.

*Methods.* Experiments were conducted in mongrel dogs of either sex, anesthetized with sodium pentobarbital, 30 mg/kg, i.v. After institution of interrupted positive pressure respiration, the thorax was opened in the midline and a strain gauge arch was sutured to the anterior aspect of the right ventricle for measurement of isometric systolic tension (4). Femoral arterial pressures were recorded from a polyethylene catheter attached to a Statham pressure transducer. Recordings were made on the Grass polygraph.

The amino acids, of highest commercial purity grade, were obtained from the Mann Research Laboratories. Except for l-arginine and l-cysteine, which were obtained as the monohydrochloride salt, the amino acids were obtained as the base. The acids were administered in Sorensen's phosphate or glycine buffer solution in volumes of 1 to 5 ml(5). In these vehicles, the amino acids were found to be relatively easily solubilized within certain specific pH ranges. These vehicles, in the pH ranges utilized in this study, failed to produce significant circulatory responses when injected into experimental animals. All doses are expressed as the base. Intravenous administration of the drugs was carried out by means of a polyethylene catheter placed in the femoral vein. Intra-aortic injections in a limited number of experiments were carried out by passing a catheter into the thoracic aorta by way of the femoral artery to the level of the diaphragm. This enabled relatively high concentrations of the amino acids to reach the adrenal glands and minimized the amount introduced into the general circulation(6). The drugs were flushed in with 5 cc of Ringer-Locke solution. Statistical analyses were carried out with the appropriate formulas(7).

*Results.* Individual amino acids generally produced biphasic or triphasic blood pressure responses, making it difficult to classify the responses into strict categories. By contrast,

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TABLE I. Neutral Amino Acids Each Exhibiting Increments in Heart Force and Mean Arterial Blood Pressure (MABP) when Injected Intravenously into Anesthetized, Vagotomized, Open-Chest Dogs.*

| Amino acid and No. of animals | Dose, mMoles/kg | pH | Mean heart force change, g ± S.E. | MABP change, mm Hg ± S.E. |
|---|---|---|---|---|
| l-leucine 7 | .0381 (7) | 11.0 ± .8 | + 58 ± 17 | +13 ± 3 |
| | .0762 (9) | 10.9 ± .8 | + 46 ± 5 | +15 ± 6 |
| | .762 (2) | 10.5 | + 90, +100 | +10, +40 |
| dl-serine 4 | .4757 (6) | 9.0 ± .5 | + 58 ± 13 | +15 ± 4 |
| | .9514 (2) | 9.6 | + 30, + 78 | +14, +30 |
| glycine 4 | .0666 (5) | 6.25 ± .10 | + 42 ± 15 | +29 ± 4 |
| l-alanine 2 | .561 (2) | 6.5 | + 10, + 20 | +16, +15 |
| | 1.122 (1) | 6.6 | + 25 | +26 |
| | 5.611 (1) | 6.5 | + 70 | +34 |
| l-cysteine 5 | .412 (1) | 8.85 | + 30 | + 4 |
| | .825 (7) | 9.3 ± .2 | + 35 ± 5 | + 7 ± 2 |
| | 1.238 (1) | 9.5 | + 45 | + 8 |
| l-threonine 3 | .209 (2) | 9.6 | + 20, + 40 | + 6, +10 |
| | .419 (2) | 9.1 | + 40, + 30 | + 8, +15 |
| | 1.049 (1) | 8.5 | +135 | +64 |
| l-methionine 3 | .335 (3) | 9.5 ± .2 | + 29 ± 6 | + 7 ± 3 |
| | 1.340 (1) | 9.5 | + 90 | +15 |
| l-serine 1 | 1.902 (1) | 7.9 | + 15 | +10 |

* Numbers in parentheses represent No. of observations.

inotropic responses were either stimulatory or depressant. Accordingly, on the basis of the major direction of the contractile force response, the amino acids were classified into two general groups: those producing primarily stimulatory responses, and those exhibiting primarily depressant responses.

All the neutral aliphatic amino acids studied in this series exhibited mostly positive inotropic effects. Table I summarizes the mean change in heart force and blood pressure produced by intravenous injection of these agents in intact animals. The mean pre-injection contractile force was 136 g force (± 20), and the mean pre-injection blood pressure value was 137 ± 30 mm Hg. A total of 31 animals were included in this series.

Positive inotropic responses were obtained with intra-aortic injections of dl-serine, l-cysteine, l-leucine, and l-methionine. Table II summarizes these intra-aortic responses. The performance of acute adrenalectomy or administration of dichloroisoproterenol (DCI, 15 mg/kg) markedly decreased the circulatory stimulant responses to intra-aortically injected l-leucine and l-methionine. Thus, the mean heart force responses to intra-aortically injected l-leucine (0.1905 mMoles/kg) and l-methionine (0.3355 mMoles/kg) were respectively + 90 ± 12 and + 94 ± 15 g change; following adrenalectomy, the heart

TABLE II. Responses to Intra-aortic Injections of Neutral Amino Acids.

| Amino acid and No. of exp | Dose (mMoles/kg*) | Mean heart force change (g ± S.E.) | Mean MABP change† (mm hg ± S.E.) |
|---|---|---|---|
| l-cysteine 2 | .4132 (2) | + 71 ± 38.6 | + 61 ± 17 |
| dl-serine 2 | .4757 (2) | + 98 ± 15 | + 11 ± 1.3 |
| l-leucine 3 | .0381 (1) | + 20 | + 25 |
| | .0762 (1) | + 40 | + 11 |
| | .7620 (1) | +145 | + 92 |
| l-methionine 2 | .3355 (2) | +110 ± 15 | +105 ± 10 |

* Numbers in parentheses indicate No. of observations.
† MABP = mean arterial blood pressure.

force responses were essentially zero. Acute bilateral adrenalectomy also decreased mean blood pressure responses from control values of 25 ± 6 and 50 ± 10 mm Hg for l-leucine and l-methionine, respectively, to values of 18 ± 6 mm Hg and 25 ± 10 mm Hg. Two experiments were conducted with each drug.

Following DCI (15 mg/kg), the mean heart force responses to l-leucine (0.3810 mMoles/kg) and l-methionine (0.6710 mMoles/kg) were decreased from + 60 ± 10 and + 120 ± 15 g to values of + 28 ± 5 and + 5 ± 3, respectively. The blood pressure response to l-leucine was also decreased from a mean value of 34 ± 6 mm Hg to 15 ± 5 mm Hg, while the pressor response to l-methionine was 105 mm Hg before and 125 ± 16 mm Hg after DCI. Three experiments were carried out with each drug.

Fig. 1 illustrates the pronounced contractile force and blood pressure responses to intra-aortically injected l-methionine. Following DCI (15 mg/kg) the contractile force response to both norepinephrine and l-methionine was virtually abolished, with persistence of most of the pressor effect.

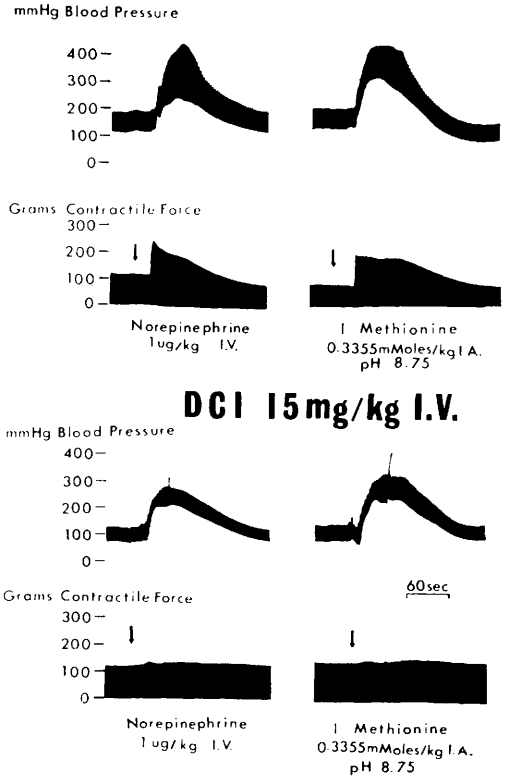


FIG. 1. Effect of DCI (15 mg/kg) on contractile force and blood pressure responses to intra-aortically (IA) injected l-methionine and intravenously (IV) injected norepinephrine: DCI given between upper and lower pair of recordings, which were separated by a time interval of 20 min.

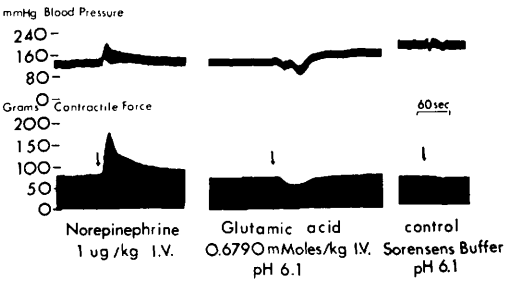


FIG. 2. Circulatory responses to injection of norepinephrine, glutamic acid and Sorensen's buffer solution.

There was a similarity in the pattern of responses between the acidic amino acids, l-glutamic acid, and l-aspartic acid and the basic amino acid, l-arginine. These amino acids were depressant to the myocardium with a minimal or absent stimulatory component following the depressant response. Blood pressure responses were biphasic; an initial depressor component being followed by a pressor response. Fig. 2 illustrates a typical response with one of these acids, glutamic acid. After injection of 0.0679 mMoles/kg of this agent intravenously, the contractile force was depressed while the blood pressure response was initially depressed and then stimulated. Table III summarizes responses obtained with each of these amino acids.

*Discussion.* The amino acids belonging to the neutral aliphatic amino acid class (l-leucine, l-serine, dl-serine, glycine, l-alanine, l-cysteine, l-threonine, l-methionine) produced positive inotropic responses, while those amino acids belonging to the acidic group (l-aspartic acid and l-glutamic acid) and basic group (l-arginine) were found to produce primarily negative inotropic responses. Cysteine, methionine, and glycine when perfused through the isolated cat papillary muscle preparation have been shown to produce positive inotropic effects(8). The relatively rapid onset in the stimulant response

TABLE III. Negative Inotropic Responses Produced by Intravenous Injection of Acidic and Basic Amino Acids in Vagotomized, Anesthetized Dogs.

| Drug and No. of exp | pH ± S.E. | Dose (mMoles/kg)* | Mean heart force change† (g ± S.E.) Decrease | Mean heart force change† (g ± S.E.) Increase | Mean MABP change† (mm Hg ± S.E.) Decrease | Mean MABP change† (mm Hg ± S.E.) Increase |
|---|---|---|---|---|---|---|
| l-glutamic acid | 5.5 ± .1 | .0679 (3) | — 8 ± 2 | — | — 3 ± 1 | + 5 ± 2 |
| 4 | 5.7 ± 1 | .6790 (3) | — 31 ±12 | + 3 ± 1 | —22 ±10 | +20 ±10 |
| | 6.1 | 2.719 (2) | —100, — 85 | — | —10, —60 | +74, +36 |
| l-arginine | 8.7 | .5740 (2) | — 4, — 2 | +20, 0 | —10, —20 | +15, +10 |
| 2 | 7.0 | 2.870 (2) | — 35, —60 | +20, +25 | —56, —18 | +10, +20 |
| l-aspartic acid | 9.5 | .1502 (2) | — 5, 0 | + 5, + 5 | —20, — 8 | + 4, + 8 |
| 4 | 9.5 ± 1 | .3756 (3) | — 20 ± 5 | +10 ± 9 | —13 ± 4 | +15 ± 6 |
| | 8.3 ± 1 | .7513 (5) | — 12 ±10 | + 7 ± 6 | — 6 ± 5 | +24 ± 6 |

* Numbers in parentheses represent No. of observations.
† Biphasic contractile force and blood pressure responses.

to these amino acids would militate against this response being due to their conversion *in vivo* to more active amines. The ability of the body to decarboxylate certain amino acids with formation of active pressor amines has been previously reported(2,9). It has been hypothesized, with only limited evidence, that this mechanism may play a role in the symptomatology found in hypertension. Thus, under conditions of renal ischemia, the kidney may actively decarboxylate amino acids to more powerful pressor amines. Leucine, for example, is converted *in vivo* to isoamyline, a more potent pressor agent which has been found to be elevated in the blood of hypertensives(10). Our study shows, however, that several amino acids are active circulatory stimulants as an inherent characteristic. The ability of DCI, an agent which blocks positive inotropic adrenergic receptor sites in the myocardium(11), to block the positive inotropic effects of l-leucine and l-methionine administered intra-aortically is compatible with the view that these agents do not directly stimulate the myocardium but may act by way of releasing catecholamines. The chief origin of these catecholamines is probably the adrenal medullae. In bilaterally adrenalectomized animals the pressor and positive inotropic effect following intra-aortic injection of several of these agents was decreased. This indicates a close interaction between the adrenal system and the blood amino acid levels.

Arginine, aspartic acid, and glutamic acid were found to be primarily depressant to the myocardium, with a slight or absent stimulant component. The blood pressure response showed both depressor and pressor components. The mechanism for the myocardial depression cannot be determined from the presently available data. Changes in pH are not involved, since control injections with varying pH ranges failed to produce negative inotropic responses equivalent to those produced by these amino acids.

*Summary.* A series of neutral amino acids (l-leucine, l-serine, dl-serine, l-cysteine, l-methionine, l-threonine, glycine) consistently produced positive inotropic and pressor responses when administered intravenously and intra-aortically in vagotomized, open-chest dogs. The ability of DCI to decrease the positive inotropic response to the intra-aortic injection of l-leucine and l-methionine indicates that adrenergic receptor sites situated in the myocardium are involved. Acute bilateral adrenalectomy also decreased these positive inotropic and pressor responses, suggesting a close interaction between the adrenal system and the circulatory stimulant effects of these agents. The acidic amino acids, l-aspartic acid and l-glutamic acid, and the basic amino acid l-arginine primarily depressed the myocardial force responses, with the blood pressure exhibiting a biphasic response.

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## EEG After Intracerebroventricular Tetrodotoxin in Cats.* (29271)

R. H. RECH, L. E. MCCARTHY AND H. L. BORISON

*Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, N. H.*

Tetrodotoxin (TT) is a potent poison extracted from the puffer fish. Systemic administration of TT causes death in mammals mainly as a result of neuromuscular paralysis(1). A direct depressant action of the toxin on vital centers in the brain stem has also been suggested(2). Borison *et al*(3) described in the cat a state of volitional paralysis following intracerebroventricular injection of TT in doses too small to cause peripheral effects. Hypothermia and respiratory depression also characterized the neurotoxic syndrome. On the other hand, TT did not appear to bring about anesthesia or analgesia since corneal, pinna, and nociceptive reflexes were not notably impaired.

This study was undertaken to examine the electroencephalographic correlates of the unusual behavioral response to TT administered intracerebroventricularly. Chronic experiments were performed in order to use unrestrained cats as their own controls and to observe concurrently the cortical and motor responses to the toxin. Acute experiments were performed on immobilized cats in order to maintain continuous artificial ventilation so as to obviate any indirect influence upon the EEG that might result from respiratory failure or other forms of motor disturbance.

*Methods. Chronic preparations.* Experiments were performed on 10 cats. The lateral cerebral ventricle was cannulated and epidural cortical electrodes were screwed into the skull under aseptic conditions in cats anesthetized with pentobarbital. In some animals a vinyl catheter was inserted into the carotid artery and secured to a plug fixed on a shoulder harness. This procedure permitted measurement of blood pressure in the unanesthetized animal. Intravenous injections were made through another catheter inserted into the external jugular vein. EEG tracings were recorded from sensorimotor and occipital cortical regions. Control recordings were taken for several days after surgery to provide representative tracings before drug treatment. The EKG was also monitored in selected animals. The crystalline toxin† was dissolved in isotonic saline and injected *via* the lateral ventricular cannula in volumes of 0.1 to 0.2 ml and total amounts of 0.1 to 0.2 μg. Effects were observed over the next 2 to 3 hours. Artificial ventilation with a Harvard pump was given as needed on emergency basis.

*Acute preparations.* Cats were prepared with cannulae, electrodes, and catheters as in the chronic preparations except that ether was employed as the anesthetic. When administration of ether was discontinued, lidocaine (1%) was infiltrated into the wound

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† Kindly supplied by Sankyo Drug Co., Tokyo.