The observed ΔZ^2 confirm this speculation. The overall reaction is presented as

$$UO_2^{2}{}_+ + 2HL^- \rightleftharpoons UO_2L_2^{2-} + 2H^+$$

The changes in ionic strength could not affect this reaction. This has been concluded from the values ($\log \beta$) which remain constant.

ACKNOWLEDGEMENT

The authors wish to thank Prof. D. D. Khanolkar, Head of the Chemistry Department, Marathwada University, for his keen interest and encouragement in this work.

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NEUROMUSCULAR EFFECTS OF PHENETHYLBIGUANIDE (DBI)*

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ABSTRACT

Activity of Phenethylbiguanide (DBI, Phenformin) has been studied on neuromuscular junction. It is shown that, DBI inhibits neuromuscular junction. Experiments to elucidate mechanism of this inhibition have shown that DBI has membrane stabilizing activity.

INTRODUCTION

IT is a long-known fact that the same structural features in different compounds are attended by similar pharmacological properties[1]. The structure of phenethylbiguanide (DBI, Phenformin), an oral hypoglycemic agent, consisting of a benzyl ring with two carbon atoms separating a biguanide radical, suggested the possibility of sympathetic action.

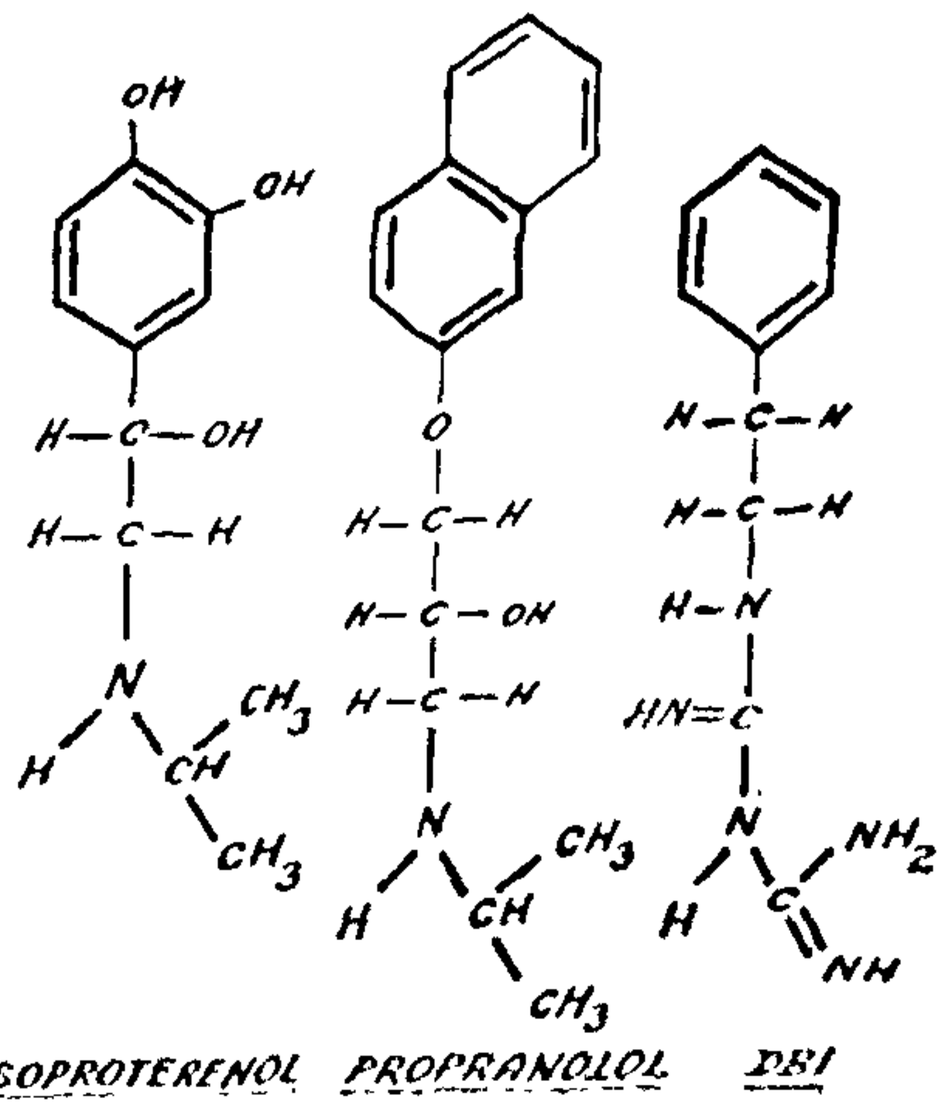
There are claims that DBI lacks a potentiating or blocking action, either to adrenaline or acetylcholine[2], and also that, only upon intravenous injection, in dogs, DBI produces an adrenergic block[3]. DBI is also reported to have negative chrontropic effect comparable to that of propranolol[4]. These contradictory reports, in spite of structural semblance of DBI with Beta-adrenergic drugs, suggested the need for critical reassessment.

There are reports that Beta-adrenergic drugs have both stimulatory[5] and inhibitory[6,7] activity at neuromuscular junction. Hence it was decided to study the activity of DBI on neuromuscular junction. Results obtained in such a study provide the basis for this report.

MATERIALS AND METHODS

(a) Frog's Rectus Abdominis Muscle

The procedure used is essentially as described by MacIntosh and Perry[8], with the following modifications. Magnification is ten fold, tension one gram; 20 to 30 min are allowed for relaxation in Frog Ringer solution (Composition, NaCl 0·65%, KCl 0·01%, $CaCl_2$ 0·012%, $NaHCO_3$ 0·01%, all W/V) before addition of physostigmine (Eserine) sulfate (2 mg/l) to sensitize the muscle. Another 20 to 30 min. are allowed for sensitization to be complete. Fifteen preparations were used for the study.



* Presented at the Second Southern Regional Conference of the Indian Pharmacological Society, Madras, October 30-31, 1974.

For acetylcholine, contractions are allowed to take place for 90 sec. The drug is then washed and the muscle allowed to relax. 90 sec. are allowed for D-Tubocurarine DBI, action after which standard dose of acetylcholine is added to the same bath without washing, and the changes in response are recorded.

(b) Frog's Gastrocnemius-Sciatic Muscle-Nerve Preparation

Method used for mounting the preparation is the same as described by Harris[9]. For both direct and indirect stimulation of the preparation, square wave pulses of 4·0 volts and 1 per 10 sec. frequency were used. Total of twenty preparations were used in 10 of which end-plate block was established using D-Tubocurarine before application of DBI.

(c) The Foot Withdrawal Reflex of the Frog

The procedure adopted is the same as described by Perry[10]. Hydrochloric acid (0·1 N) was used as stimulus. Experiment was repeated in 12 preparations.

(d) Surface Anaesthesia in Rabbits

Surface anaesthesia in rabbits is tested as described by Weatherby[11]. A group of ten rabbits were used for the study.

Results

(a) Frog's Rectus Abdominis Muscle:

DBI had a dose-proportionate inhibitory effect on acetylcholine induced contracture in all the preparations used. A period of 90 sec. of DBI contact with the preparation was sufficient to produce this inhibition. At least 20–30 min. waiting, with repeated washing was necessary to bring back the preparation to its pre-inhibitory responsive state (Fig. 1 *a*). By itself, DBI did not stimulate the preparation. A similarity of its action, as compared with D-Tubocurarine and Propranolol, can be seen in Fig. 1 *a*. Combination of DBI and D-Tubocurarine produced additive effect (Fig. 1 *b*).

(b) Frog's Gastrocnemius-Sciatic Muscle-Nerve Preparation

Local application of DBI on nerve decreased the response of indirect stimulation, when it was done, proximal to drug application. Response to indirect stimulation distal to drug application was unaltered (Fig. 2 *c*).

Injection of DBI (0·2 to 0·3 ml of 1% solution) to muscle belly produced a total block for both indirect and direct stimulations. This blockade was not reversed by Neostigmine.

In curarised preparations with end plate block, (*i.e.*, response to indirect stimulation absent and response to direct muscle stimulation present) injection of DBI to muscle belly abolished the response to direct stimulation also (Fig. 2 *d*).

(c) The Foot Withdrawal Reflex of the Frog

Absence of withdrawal reflex was seen after an average of 6–8 min. when 1% solution of DBI was used.

(d) Surface Anaesthesia in Rabbits

Effects of 1·0% solution of DBI after local application was observed for a period of 2½ hours.

DBI produced loss of corneal reflex and slight mydriasis. Light reflex was unaffected. Onset of loss of corneal reflex was on an average of 20–25 min. Corneal reflex did not reappear at the end of 2½ hours.

Discussion

Acetylcholine response of frog's rectus abdominis muscle is blocked by DBI. Effects of D-Tubocurarine and DBI are synergistic.

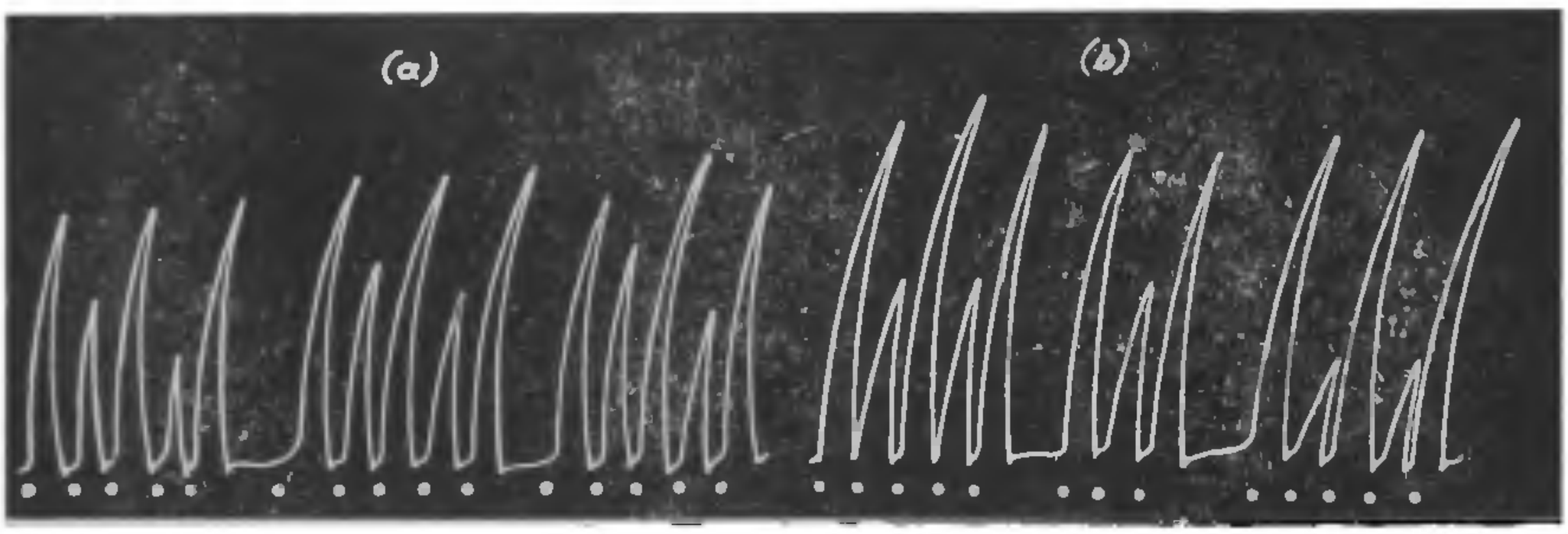


Fig. 1. Frog's rectus abdominis muscle preparation. (*a*) DBI showing inhibition of acetylcholine induced contracture. (*b*) Effect of combination of DBI and D-Tubocurarine on acetylcholine induced contracture. A, Acetylcholine 0·2 mcg. in (*a*) and 0·8 mcg. in (*b*); D–T, = D–Tubocurarine, 1·0 mcg, in (*b*); *p*, propranolol.

Responses to indirect and direct muscle stimulation in frog's gastrocnemius-sciatic muscle-nerve preparation are also blocked.

DBI has produced local anaesthetic action.

Mechanism of neuromuscular blocking action of DBI may be due to (1) Curare like—antidepolarising action. (2) Succinylcholine like—persistent depolarization action. (3) Membrane stabilization.

Unlike curare if also blocks direct muscle action and its action is not antagonized by neostigmine. Absence of initial contraction with DBI and abolition of response to direct stimulation of muscle precludes persistent depolarization mechanism.

The presence of local anaesthetic action with DBI and a similarity to propranolol action on neuromuscular junction stresses the possibility of a direct membrane stabilization action to DBI.

A comparison of DBI and propranolol is given in Table I. A number of points of similarity can be noted.

TABLE I

Some similarities between propranolol and DBI

Properties	Propranolol	DBI
1. Structural similarity	Present	Present
2. Hypoglycemia	Present[12]	Present[13]
3. Membrane stabilization		
(*a*) Skeletal muscle Membrane	Present[5]	Present**
(*b*) Cornea	Present[11,15]	Present**
(*c*) Negative chronotropic effect	Present[16]	Present[11]

** — Finding of the present study.

These similarities between Beta-adrenergic blocking drugs and DBI, warrant further study of DBI.

ACKNOWLEDGEMENTS

The authors wish to thank Mr. U. J. Devadiga, of Kasturba Medical College, Manipal, for his help in preparing the illustrations.

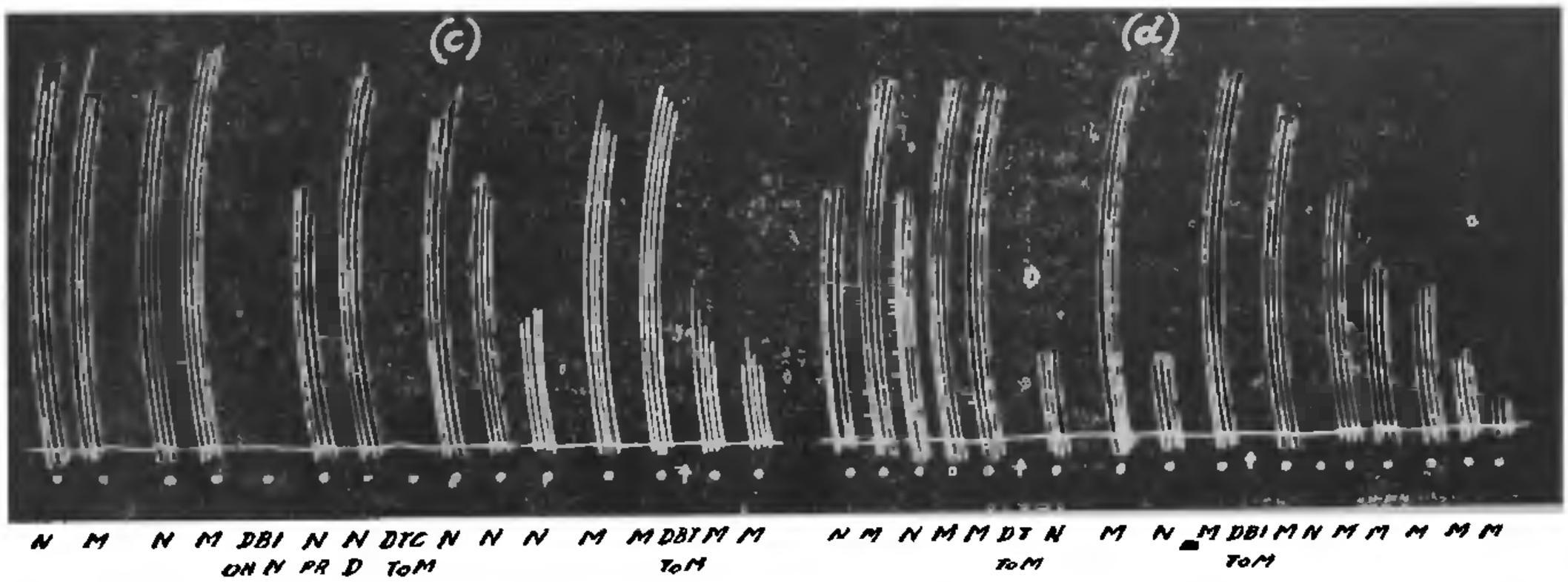


FIG. 2. Effect of DBI on frog's gastrocnemius—Sciatic muscle—nerve preparation. (*c*) DBI showing membrane stabilization at the point of application. (*d*) DBI Showing inhibition of direct muscle stimulation in curarised preparation. N, Nerve stimulation; M, Muscle stimulation DBI, ON N, Application of DBI on nerve, D. TCTO M, Injection of D–Tubocurarine to muscle belly; DBI TO M, Injection of DBI to muscle belly; N PR, Nerve stimulation proximal to drug application; N D, Nerve Stimulation distal to drug application.

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POLAROGRAPHIC STUDY OF MIXED LIGAND COMPLEXES: CADMIUM(II)-THIOUREA-FORMATE SYSTEM

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ABSTRACT

Cd(II)-thiourea-formate system was investigated polarographically in aqueous medium at 25 ± 0·5° C. The ionic strength was maintained at 2·0 M with potassium nitrate. Schaap and McMasters treatment of experimental data revealed the existence of $[Cd(tu)\ (fir)_2]$ and $[Cd(tu)_2\ (for)]^+$ with their respective stability constants as $\log \beta_{11} = 3{\cdot}65$ and $\log \beta_{21} = 3{\cdot}99$. The negative value of β_{11} indicates the absence of $[Cd(tu)\ (for)]^+$ complex in solution.

INTRODUCTION

ALTHOUGH, most of the studies reported in literature have been made with the help of potentiometric[1] and spectrophotometric[1,2] techniques, the determination of the nature of mixed ligand complex formation by polarographic method has been receiving considerable attention in comparatively recent times[3]. This paper deals with the study of the composition and the stability constants of mixed ligand complex species which are formed by cadmium (II) with formate and thiourea. The simple systems, namely, Cd (II)-formate[4,5] and Cd (II)-thiourea[6,0] were previously studied polarographically at different ionic strengths and in aqueous and non-aqueous media. However, these simple systems were reinvestigated in the present work in order to obtain stability constants of complexes under identical conditions maintained for mixed systems.

EXPERIMENTAL

Potassium nitrate was used as a supporting electrolyte to maintain the ionic strength at 2·0 M in all cases. Triton X-100 (0·004%) was used as a maximum suppressor. The pH of the solutions to be polarographed was in the range of 6·6 to 6·8. A Cambridge Pen Writing Polarograph was used to record the polarograms. Purified nitrogen was bubbled to exclude the dissolved oxygen. All half-wave potentials refer to saturated calomel electrode (S.C.E.). The dropping mercury electrode had the following characteristics:

$t = 3{\cdot}0$ sec in 0·1M KNO_3 (open circuit)
$m = 1{\cdot}97$ mg sec^{-1}, $m^{2/3} \times t^{1/6} = 1{\cdot}88$ $mg^{2/3}$ $sec^{-1/2}$

The measurements were carried out in an air conditioned room where the temperature was maintained at 25 ± 0·5° C.

RESULTS AND DISCUSSION

The Stability Constants of Cd (II)-Thiourea System:

All the solutions contained 1mM Cd (II) and 0·004% Triton X-100. The concentrations of thiourea varied from 0·05 to 0·6 M. The half-wave potential of Cd (II) (2·0 M KNO_3) was −0·593 V which shifted to more negative potential by the addition of thiourea showing the complex formation.

The relationship between $-(E_{\frac{1}{2}})_c$ and log C_L, where C_L is the concentration of thiourea (tu), was not linear but gave a smooth curve with three segments indicating the existence of 1:1, 1:2 and 1:3 complex species. The method of DeFord and Hume[10] was, therefore, applied to evaluate the stability constants which were found to be as under:

$\log\beta_{10} = 1{\cdot}69 \pm 0{\cdot}11$, $\log\beta_{20} = 1{\cdot}95 \pm 0{\cdot}10$ and $\log \beta_{30} = 3{\cdot}47 \pm 0{\cdot}03$. Our values are nearly identical to those observed by Lane *et al.*[6] and Migal *et al.*[7,8] The slight divergence may be due to the differences in the ionic strengths and the media used.