PYRIDINE NUCLEOTIDES : BASIS FOR THE HYPOTENSIVE ACTION OF TRIPHOSPHOPYRIDINE NUCLEOTIDE

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Summary: Intravenous administration of TPN produced an abrupt, transient, reproducible and dose-dependent fall in systemic blood pressure of urethane anaesthetised rats, rabbits and cats. In guinea pigs the effect was found to vary from animal to animal. Whereas in some guinea pigs, TPN provoked biphasic, pressor-depressor response; in others either of the pressor or depressor responses was obtained. Vagosympathectomy either abolished or reduced the pressor response but depressor response remained unaltered in guinea pigs. Hypotensive response persisted in pithed rats and remained unaltered in rats after β-adrenoceptor, histaminic receptor and ganglionic blockade. Atropine antagonized the hypotensive response to TPN and physostigmine potentiated it. Antagonism by atropine was, however, more with lower doses of TPN and responses to its relatively higher doses were partially antagonised. Left vagotomy failed to alter the response to TPN but right vagotomy antagonized and bilateral vagotomy further reduced it. Further reduction in depressor response to TPN in bivagotomized rats by atropine indicated involvement of extravagal cholinergic component. Cumulative administration of TPN reduced the pressor effect to adrenaline and depressor effect of histamine for a short while but failed to alter hypotensive response to acetylcholine.

Key words: TPN depressor response cholinergic

INTRODUCTION

Pyridine nucleotides have been found in all the cells examined and are responsible for numerous oxidation—reduction reactions in the cell metabolism including the energy synthesizing and utilizing systems. These nucleotides influence cellular redox, which has, recently been implicated in factors governing the cellular and tissue response to various drugs. It has been suggested that a tissue reaction to drugs depends upon its 'metabolic structure' i.e., upon whether the Embden-Meyerhof-Kreb's system or the pentose pathways are predominant (5). Modes of action of various drugs such as nicotine, strychnine, chloroform, thiopentone, ether, pentetrazole, etc. have been interpreted and fitted into the theoretical framework of this hypothesis by Laborit (5) and Laborit and Weber (6). Based on the availability of such data in literature, we considered it worthwhile to look for pharmacological effects of pyridine nucleotides. In previous experiments, it was communicated that triphosphopyridine nucleotide (TPN) produced transient hypotensive response in rats (2). Attempts have been made to define the mechanisms underlying the vasodepressor actions of TPN. The aim of this paper is to report the effect of TPN on blood pressure in different laboratory animals and present data obtained from experiments designed to search for

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the site of action of this nucleotide in rat.

MATERIALS AND METHODS

Male albino rats weighing 150 to 250 *g* purchased from Haffkine Institute, Bombay, guinea pigs (400 to 600 *g*), rabbits (1 to 2 *kg*) and cats (2 to 4 *kg*) of either sex obtained from commercial sources were used. The animals were anaesthetised by urethane (1.4 *g/kg*) and not artificially ventillated till otherwise mentioned. Carotid artery blood pressure was recorded on smoked paper of a rotating drum. Drugs were injected through a cannula into the femoral vein and all the animals were heparinized before use.

(*i*) *Normal animals:* Graded amounts of freshly prepared solutions of TPN were injected into the femoral vein to obtain response in relation to dose. Reproducibility of response to the nucleotide was ascertained by repeated administration of TPN for 5-6 *hr*. Injections were flushed in with saline and care was taken to keep the speed of the injection constant.

(*ii*) *Vagotomized rats:* Experiments were designed to assess involvement of right and left vagus individually. Rats were divided into two groups. After recording normal response to graded doses of TPN in one group, the left vagus was sectioned at the cervical region and then after determining response to same doses of the nucleotide, bivagotomy was achieved by sectioning the right vagus. Response to TPN was again determined. In another group, right vagotomy was first performed and then bilateral vagotomy was achieved.

(*iii*) *Atropine treated rats:* After obtaining response with graded doses of TPN response to acetylcholine (Ach) was recorded and then atropine 1 *mg/kg* (im) was injected. Response to TPN was obtained after ascertaining the extent of block of hypotensive response to Ach. Similarly, the effect of atropine on response to TPN was assessed in bilateral vagotomized rats.

(*iv*) *Pithed rats:* Male rats weighing 200-300 *g* were prepared according to the method described by Shipley and Tilden (7). Dose response relationship was studied with TPN and the average per cent fall in blood pressure was compared with the average fall at equal doses in intact rats. For the purpose of comparison, since initial blood pressure in pithed rats ranged between 80 and 100 *mm Hg*, data obtained from normal intact rats of relatively lower basal blood pressure were used.

(*v*) *Physostigmine-treated rats:* Response to 3 graded doses of TPN was obtained before and after physostigmine treatment which was achieved by injecting it in a dose of 3 *mg*/100 *g* intravenously three times with an interval of 1 min between each injection and assured by potentiation and prolongation of the hypotensive response to 1 $\mu g/kg$ of Ach.

(*vi*) *Mepyramine-treated rats:* After recording response to graded doses of TPN, mepyramine 3 *mg/kg* was injected intramuscularly and then after ascertaining block of the hypotensive action of 1 $\mu g/kg$ histamine, TPN was again administered.

(*vii*) *Pronethalol-treated rats:* Pronethalol was injected intramuscularly 10 *mg/kg* and

sponse to graded doses of TPN was obtained before and after pronethalol administration.

(*viii*) *Pentolinium-treated rats:* The effect of TPN was observed in rats before and after mg/kg of pentolinium administration.

(*ix*) *Rat hind leg quarters preparation:* Rat hind leg preparation was set up according to the method of Burn (1) to test whether TPN had any peripheral vasodilation action. Injections were made into the small glass chamber with rubber cap, immediately before the cannula tied into the aorta. Effluent drops leaving the vein were recorded through a magnetic flow recorder and automatic drop counter.

(*x*) *Effect of TPN on adrenaline (A) and histamine response:* It was observed during the course of experiments that, in rats, which had previously been subjected to cumulative administration of the coenzyme, response to A and/or histamine was reduced or even abolished. To examine this observation, in some rats after recording two responses to 1 $\mu g/kg$ of A or histamine, rats were administered with a series of TPN injections and then response to the same dose of the amine was observed. Studies on histamine and A were carried out in different animals.

Results have been presented as % fall in blood pressure for comparisons, since the magnitude of fall produced depended on the initial basal blood pressure level. Differences in the mean responses observed were tested for significance by 'paired test statistics' if the responses were from the same animal. Whenever data from different animals was to be compared 'unpaired test statistics' was applied (3) . Concentrations of drugs cited in the text refer to their respective free bases.

Drugs: Acetylcholine (E Merck) histamine acid phosphate (BDH), TPN (A grade, Sigma Chemicals), mepyramine maleate (BDH), pentolinium tartrate (Ansolysin, May & Baker), physostigmine (Eserine T. H. Smith Ltd.). atropine sulphate (T. H. Smith Ltd.), Pronethalol (Alderlin I.C.I.), urethane ethyl carbonate (BDH), heparin (Biological Evans), l-adrenaline tartrate (Parke Davis).

RESULTS

(*i*) *Normal anaesthetized animals:* Intravenous injection of TPN produced an abrupt and transient fall in the arterial blood pressure of urethane anaesthetized rats, rabbits and cats (Table I). Qualitatively, the response was similar in all the species of animals and was found to be dose-dependent and reproducible without any evidence of tachyphylaxis. Basal blood pressure level remained more or less unaffected by TPN up to the end of the experiment. In some rats, hypotensive response was followed by a small rise in blood pressure which did not show any definite pattern of dose-relation.

In guinea pigs, TPN provoked a biphasic, pressor-depressor response in about 50%of the animals. However, in the rest of the guinea pigs, either pressor or a depressor response was observed. Both the responses were abrupt and transient. Typical responses are shown in Fig. 1 (a)

TABLE I: Effect of TPN on mean arterial blood pressure.

Dose of TPN μg/kg	*Mean fall in B.P. (mm Hg) ± S.E.M.*					
	Rat		*Rabbit*		*Cat*	
25	11.8 ± 1.09	(20)	16.3 ± 3.66	(9)		
50	24.0 ± 2.11	(20)	26.9 ± 4.87	(9)	14.0 ± 3.18	(6)
100	36.0 ± 2.16	(20)	39.6 ± 5.63	(9)	20.0 ± 3.93	(6)
200	47.8 ± 1.92	(20)	47.6 ± 8.37	(9)	24.0 ± 3.8	(6)

Figures in parenthesis denote the number of experiments. TPN was injected into the femoral vein. For other details see text.

and 1 (b). Whereas hypotensive response showed a definite dose-response relationship, hypertensive response did not. Pressor effects were reduced after repeated administration, suggesting development of tachyphylaxis.

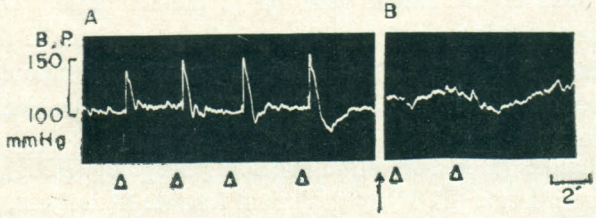

Fig. 1(a): Transient hypertensive response to (from left to right) 50, 100, 200 and 400 μg/*kg* of TPN before (A) and to (from left to right) 200 and 400 μg/*kg* of TPN after (B) sectioning of vagosympathetic trunk, in a ginea pig. Note the disappearance of the pressor response (B). TPN was injected at triangles.

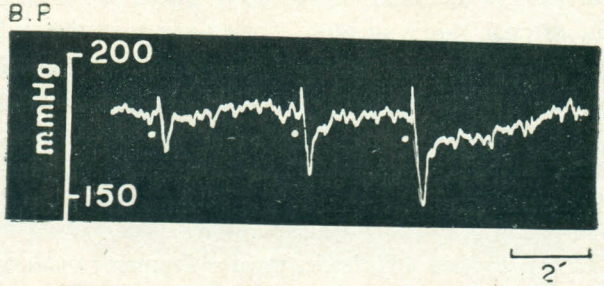

Fig. 1(b): Transient hypotensive effect in response to (from left to right) 50, 100 and 200 μg/*kg* of TPN in another guinea pig. TPN was injected at dots.

Sectioning of the vago-sympathetic trunk either abolished or reduced the pressor response to PN in guinea pigs, but the depressor response remained unaffected.

(*ii*) *Vagotomized rats:* Scissoring of the left vagus nerve did not affect ($P>0.05$) the hypo-sive response to TPN but right vagotomy reduced it significantly ($P<0.05$) and bilateral vagoto-y further decreased but failed to abolish the response (Table II). The nature of fall in blood pre-ure remained unaltered except that in some rats the onset of action was slightly delayed and the ration of action was prolonged.

TABLE II: % change (fall) ± S.E.M. in rat blood pressure by TPN under different treatments.

Treatment		*Dose of TPN μg/kg* 50	100	200	*No. of animals*
Left vagotomy	before	21.1 ±4.04	25.5 ±3.21(NS)	31.0 ±2.95	(10)
	after	20.1 ±4.33	26.4 ±2.56	33.8 ±2.36	
		P >.05	P >.05	P >.05	
Right vagotomy	before	19.7 ±2.92	25.1 ±1.83	31.4 ±1.62	(9)
	after	14.8 ±3.04	17.0 ±2.45	23.2 ±2.46	
		P >.05	P <.05	P <.05	
Bilateral vagotomy	before	17.9 ±1.71	24.1 ±1.31	29.1 ±1.79	(10)
	after	13.1 ±1.43	13.2 ±1.79	17.7 ±2.2	
		P <.05	P <.01	P <.01	
Physostigmine	before	18.4 ±1.62	24.6 ±1.26	30.6 ±1.28	(8)
	after	23.5 ±1.71	29.8 ±1.64	44.6 ±3.86	
		P <.01	P <.05	P <.01	
Mepyramine	before	19.4 ±2.69	25.01 ±2.71	31.8 ±2.79 (NS)	(8)
	after	18.5 ±4.51	23.5 ±4.02	32.1 ±4.17	
		P >.05	P >.05	P >.05	
Pronethalol	before	18.3 ±2.05	24.6 ±2.22	29.7 ±2.37 (NS)	(8)
	after	19.2 ±2.24	23.9 ±1.84	29.3 ±2.64	
		P >.3	P >.2	P >.3	
Pithing*	Control	17.2 ±1.99	23.32 ±2.67	29.21 ±2.91	(6)
	pithed	17.04 ±1.55	23.33 ±2.36	29.16 ±2.72 (NS)	
		P >.2	P >.3	P >.3	
Pentolinium*	Control	17.2 ±1.99	23.32 ±2.67	29.21 ±2.91	(10)
	treated	16.32 ±1.84	18.72 ±2.53	24.05 ±2.23 (NS)	
		P >.3	P >.1	P >.05	

*Unpaired 't' test was used to obtain significance of difference; for details see text.

(*iii*) *Atropinized rats:* Hypotensive response to TPN was markedly antagonised or abolished after atropine administration was sufficient to abolish the depressor effect to 1 $\mu g/kg$ of Ach. Out

of 24 rats, in six the hypotensive response to 200 $\mu g/kg$ of TPN was abolished, in eight rats it was anatagonized above 70%, in two above 60% and in four above 55%. The response in the remaining four rats was affected slightly. The extent of blockade of depressor response was more in the case of relatively lower doses of TPN (50, 100/$\mu g/kg$); when relatively higher doses (500/μg to 2 mg/kg) of TPN were administered blockade varied largely from animal to animal and was always partial. In bivagotomized rats, atropine further reduced the response to TPN suggesting involvement of extravagal cholinergic mechanisms. However, atropine blockade of the depressor response was far less in bivagotomized than in normal rats.

(*iv*) *Pithed rats:* TPN produced an immediate transient hypotensive response in pithed rats. Pithing rendered the rats hypotensive with blood pressure levels ranging between 80 and 120 *mm Hg*. (mean of 96.4±6.25 *mm Hg*). Taking into account the observation that magnitude of fall by TPN depended upon the initial basal pressure level of the animal, hypotensive response to TPN in pithed rats and in normotensive rats was not compared. But, data obtained from a group of normal rats with spontaneous low blood pressure (basal pressure, 84 *h* 120 *mm Hg*). was used for comparison. The difference in response was insignificant ($P>0.1$) between these groups of rats suggesting that pithing did not markedly influence the hypotensive response to TPN.

(v) *Physostigmine-treated rats:* Physostigmine treatment potentiated the hypotensive response to TPN significantly ($P<0.05$) in its magnitude and duration. Results obtained from typical experiment are shown in Fig. 2 and the data obtained from other animals are listed in Table II. The Mean basal blood pressure level increased after physostigmine from 140.0±8.96 to 148.7±10.86 *mm Hg*.

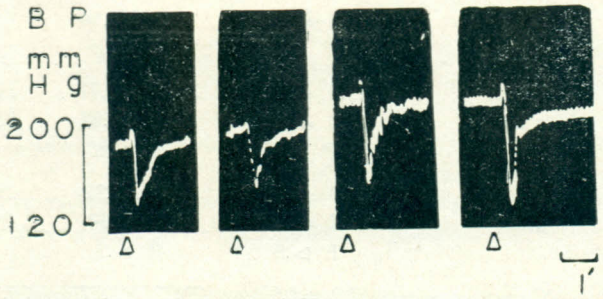

Fig. 2: Potentiation of the hypotensive action of TPN in a rat after physostigmine treatment. Tracings are in response to 100 $\mu g/kg$ TPN (A), 1 $\mu g/kg$ Ach (B) before and 1 $\mu g/kg$ Ach (C) and 100 $\mu g/kg$ TPN (D) after physostigmine treatment. Note potentiation of responses to Ach, (B to C) and to TPN (A to D). Drugs were injected at triangles.

(*vi*) *Ganglion bockade:* Ganglion blockade by pentolinium rendered the rats hypotensive with a mean basal blood pressure of 94.2±7.45 *mm Hg* without affecting the per cent fall by TPN significantly ($P>0.1$). Responses to TPN in pentolinium treated rats were compared with the normal group of rats with relatively low blood pressure as detailed in the case of pithed rats.

Neither mepyramine nor pronethalol tratment affected the depressor reponse to TPN in rats Table II).

(*viii*) *Response to A and histamine:* Cumulative administration of TPN (total 1 to 2 g/kg) influenced the pressor response to A and the depressor response to histamine. The first jection of the test dose of A after TPN administration failed to induce any pressor response in 3 periments. Under similar circumstances the response to histamine was either antagonized or duced. However, such a type of TPN did not persist and further injection and histamine showed tendency for recovery towards their normal responses and after four to five injections, there as complete restoration of the response. It should be emphasised that TPN could exert such fects only if the amines were injected soon after the nucleotide administration. Surprisingly e response to Ach remained unaffected by prior treatment with TPN.

(*ix*) *Rat hind leg preparation:* TPN (5 to 500 μg) did not alter the venous outflow from e perfused rat hind leg. At relatively high doses (1 *mg*—3 *mg*) however, there was slight but in-nsistent vasodilatation.

DISCUSSION

TPN produced a transient, reproducible, dose-dependent fall in arterial blood pressure of ure-hane anaesthetized rats, rabbits and cats. Construction of dose-response curves revealed TPN o be less effective in cat and equally effective in rat and rabbit. Preliminary attempts to establish achyphylaxis failed. In guinea pigs the response varied from animal to animal. Whereas, it as purely pressor in some animals, in others a biphasic response was obtained.

Atropine blockade of the hypotensive response to lower doses of TPN in rats suggested choli-nergic mediation of the response. This observation was further substantiated by potentiation of he hypotensive response by physostigmine treatment. In higher doses however, TPN seemed to exert its effect through other mechanisms, since it was slightly affected by atropine. Diminution of the hypotensive response after transection of the bilateral vagus nerve suggests that TPN might involve a reflex action originating in the heart with afferent pathways in vagal nerve fibres. Another interesting observation in this study was a profound reduction of the hypotensive response to TPN by unilateral right vagotomy. An extravagal cholinergic component was revealed by the observa-tion that in bilateral vagotomized rats, atropine would further reduce the hypotensive response to TPN.

The persistence of the hypotensive response to TPN in pithed rats suggests that central action may not be involved in the nucleotide-induced transient hypotension. Similarly interruption of the flow of impulses through sympathetic ganglia due to ganglion blockade did not significantly affect the response. The involvement of histamine receptors and β-adrenoceptors was ruled out since the response remained unaltered after blockade of these sites. TPN also failed to increase the venous outflow from the hind quarter of the rat in doses which produced profound hypotension.

No explanation for the transient antagonism of A and histamine response by TPN can be given at this stage. Only some assumption on the basis of role of cellular environment in directing the response to a drug (5, 6) could be made. One of the TPN-induced metabolic alteration in the cell may in some way interrupt the interaction between the amines and their respective receptors and this metabolic alteration could be overcome by repeated administration of these amines. Even a chemical antagonism in the presence of cellular environment between TPN and these amines cannot be ruled out.

The mechanism of the response to TPN in guinea pigs forms a part of our future investigation. The profound reduction of the pressor response after sectioning of the vagosympathetic trunk, could, however, involve several mechanisms. It may be, either, a central action due to increased sympathetic discharge like that of angiotensin (8) or a peripheral vasoconstricting effect of myogenic or neurogenic component through sympathetic nerve (9). The occurrence of tachyphylaxis to the pressor effects of TPN by its repeated administration may, however, involve the release of sympathomimetic amines from some stores.

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