POTENTIATION OF OXYTOCIN AND PROSTAGLANDINS- EVOKED RESPONSES BY (+) INPEA ON ISOLATED RAT UTERUS: ITS SPECIFICITY AND SELECTIVITY

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Abstract—The effect of (+) INPEA on various stimulant drugs was examined on isolated rat uterus. Addition of (+) INPEA (1×10^{-8} g/ml) to the organ bath, produced marked potentiation in the contractile responses of oxytocin and prostaglandins. Potentiation was less significant to 5-HT, vasopressin, angiotensin and bradykinin. (±) INPEA did not potentiate the responses of oxytocin on isolated rat mammary strip and the responses of prostaglandins on rat stomach (fundus) strip, guinea-pig tracheal chain and guinea-pig ileum. The significance of these findings has been discussed.

(±) INPEA (1-4'-nitrophenyl-2-isopropyl aminoethanol) hydrochloride, an adrenergic beta receptor blocking agent has been clinically evaluated in angina pectoris and cardiac arrhythmias and was shown to be effective (1, 2). Its beta blocking effect has been reported to be considerably less than the reference beta blocker, propranolol (3). While studying the pharmacological properties of INPEA, Saini and Sharma (4) observed a marked difference in the pharmacological activity between its optical isomers. Both (−) INPEA and (±) INPEA exhibited weak antioxytocin activity. In contrast, (+) INPEA produced a marked potentiating effect of oxytocin-evoked responses. In subsequent studies, (−) INPEA also markedly potentiated prostaglandins-evoked responses of isolated rat uterus (5). Recently, a significant sensitization to oxytocin and prostaglandins-evoked responses was observed in uteri of rats pretreated with (+) INPEA (6).

In the present study a number of uterine stimulants and a number of tissues responsive to oxytocin or prostaglandins were used to investigate the specificity and selectivity respectively, if any, of the (+) INPEA-induced potentiation.

MATERIALS AND METHODS

Specificity of (±) INPEA-induced potentiation

The potentiating effect of (±) INPEA on the contractile responses evoked by various agonists was studied on isolated rat uterus preparation, as described by Holton (7). Oestrus induced rats (oestradiol dipropionate 200 µg, s.c., 15 hr prior to starting the experiment) of Charles-Foster strain weighing 150-200 g were used. The animals were stunned by a blow on the head and a small piece (2 cm) of the cervical end of the uterine horn was removed and suspended in an organ bath (10 ml) containing de Jalon's solution (9 g NaCl,

0.42 g KCl, 0.06 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.5 g glucose, 1 g NaHCO_3 , 0.05 g NaH_2PO_4 , distilled water added to make 1000 ml., all reagents A.R.) at 29°C which was aerated through an air pump (C.F. Palmer, London; Model ES-ES). The contractions were recorded on a Servoscribe potentiometric recorder (Smiths Industries Ltd., model RE 511.20) using a force displacement transducer (Grass Instrument Co. FT-03). The resting tension on the tissue was 0.5 g. An equilibration period of 30 min was allowed before the addition of drugs. After stabilization, no spontaneous change in excitability of uterine tissue was noticed in control experiments over an observation period of 120 minutes.

Once the tissue was stabilized, control responses to (A) a single dose and (B) graded doses of each of the agonists were obtained at intervals of five minutes. The drug was allowed to be in contact with the tissue for 45 sec and two changes in the bath fluid were made with fresh de Jalon's solution in the intervals between doses. While taking responses for a single dose, to avoid approximation of the potentiated response to the maximal response, the dose which gave the minimal detectable response was selected for each drug. For obtaining the dose-response, graded doses were continued until the maximal response was reached. After obtaining control responses, (—) INPEA (1×10^{-5} g/ml) was added to the de Jalon's solution and allowed to act for 30 min after which time the doses of the agonists were repeated. At this concentration (based on preliminary experiments), (+) INPEA did not, per se, induce spontaneous contractions and also produced a maximal effect on oxytocin and prostaglandins-evoked contractions. The various agonists used were: oxytocin, prostaglandins (PGE_1 , PGE_2 and $\text{PGF}_{2\alpha}$), vasopressin, angiotensin, 5-HT and bradykinin.

From the tracings obtained in the above experiments, (+) INPEA-induced potentiation was analysed by measuring the heights of contractions before and after (+) INPEA with each of the agonists studied. In the experiments wherein the maximal responses were obtained with each agonist, log-dose response curves were constructed and ED50 (the effective molar concentration of the drug necessary to produce 50% of the maximal response) values were determined and the values obtained were compared before and after the addition of (—) INPEA.

Selectivity of (—) INPEA-induced potentiation

Experiments were carried out on the following preparations to ascertain whether (—) INPEA-induced potentiation is selective only for the uterine tissue or is non-selective and occurs in other tissues as well. The most specific agonist for that particular tissue was used in these experiments.

Isolated strip of rat mammary gland: The method of Reyden and Sjöholm (8) was followed. Lactating rats (3–10 days after delivery of litters) were sacrificed and one of the lower mammary glands was removed. A strip (3 cm $\times$ 0.5 cm $\times$ 0.5 cm) was suspended in a 10 ml organ bath at 38°C containing Tyrode's solution (NaCl 8 g, KCl 0.2 g, CaCl_2 0.15 g, NaHCO_3 1 g, glucose 1 g, MgCl_2 0.1 g, NaH_2PO_4 0.05 g, distilled water added to make 1000 ml). The solution was saturated with a mixture of 95% O_2 and 5% CO_2 . A 0.5 g tension was applied to the tissue and an equilibration period of one

hr was allowed before the addition of drugs. Contractions evoked by the graded doses of oxytocin were recorded at intervals of 10 min by a potentiometric recorder. The drug was allowed to be in contact with the tissue for 60 sec and two changes of the bath fluid were made in the intervals between doses.

After obtaining the control responses, (-) INPEA (1×10^{-5} g/ml) was added to the bath fluid and allowed to act for 30 min after which time the doses of oxytocin were repeated. Log-dose response curves were constructed and a change in the ED50 value produced by (-) INPEA was noted.

Isolated stomach (fundus) strip of rat: The method used was that of Vane (9). Rats which were fasted overnight were sacrificed and the fundic part of the stomach was removed. The tissue was transferred to a dish containing Krebs's solution (NaCl 5.54 g, KCl 0.5 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.2 g, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.28 g, KH_2PO_4 0.16 g, NaHCO_3 2.1 g, glucose 2.1 g, distilled water added to make 1000 ml). A 4.5 cm long strip was made from each of the stomachs by suitable transverse cuts and suspended in an organ bath (10 ml) containing Krebs's solution at 37°C and was aerated through Palmer's aeration pump. A resting tension of 1 g was applied to the tissue. After an equilibration period of 1 hr, responses to graded doses of PGE_1 were taken at intervals of 15 min on a slowly moving smoked drum using an isotonic lever which gave an 8 fold magnification. The drug was allowed to be in contact with the tissue for 60 sec and at least two changes in the bath fluid were made in the intervals between doses. Log-dose responses were constructed and the change in the ED50 value produced by (+) INPEA was noted.

Isolated guinea-pig ileum: The method used was that of Magnus (10). Adult guinea-pigs weighing above 300 g were sacrificed and a small piece (3–4 cm) of ileum was suspended in the isolated bath of 10 ml capacity filled with Tyrode's solution at 37°C and was aerated. A resting tension of 0.5 g was applied to the tissue. After an equilibration period of 1 hr, responses evoked by the graded doses of PGE_2 were recorded on a potentiometric recorder. The drug was allowed to be in contact with the tissue for 30 sec and a 15 min interval between doses followed to avoid any possibility of developing tachyphylaxis. At least two changes in the bath fluid were made in the intervals between doses.

After obtaining the control responses of PGE_2 , (+) INPEA (1×10^{-5} g/ml) was then added to the bath fluid and allowed to act for 30 min after which time the same doses of PGE_2 were repeated. Log-dose response curves were plotted and the change in the ED50 value produced by (-) INPEA was noted.

Isolated guinea-pig tracheal chain: The method of Castillo and de Beer (11) was followed. Adult guinea-pigs were sacrificed and the trachea was dissected and transferred to a dish containing Krebs's solution. The trachea was then sectioned into rings by cutting transversely between the segments of the cartilage. Six to seven rings were tied together to form a chain with the muscular part of the rings arranged alternately. The chain thus prepared was suspended in an organ bath (10 ml), containing Krebs's solution at 37°C which was saturated with a mixture of 95% O_2 and 5% CO_2 . An initial

tension of 0.2 g was applied to the tissue. After an equilibration period of 1 hr, responses to graded doses of $\text{PGF}_{2\alpha}$ were taken at intervals of 15 min on a smoked drum using a straw lever which gave an 8 fold magnification. The drug was allowed each time to be in contact with the tissue for 60 sec and at least two washings were made in the intervals between doses.

After obtaining control responses to $\text{PGF}_{2\alpha}$ (---) INPEA (1×10^{-5} g/ml) was added to the bath fluid and allowed to act for 30 min after which time the doses of $\text{PGF}_{2\alpha}$ were repeated. Dose-response curves were plotted and the shift in the curve produced by (---) INPEA was noted. Drugs: Oxytocin (1 mg = 400 I.U.; Syntocinon, Sandoz); prostaglandin $\text{F}_{2\alpha}$ tromethamine, prostaglandin E_1 and prostaglandin E_2 (Upjohn); arginine vasopressin (1 mg = 400 I.U., Sandoz); angiotensin (Hypertensin, Ciba); bradykinin (Parke-Davis); 5-hydroxytryptamine creatinine sulphate (May and Baker); (---) INPEA hydrochloride (Selvi & Co).

Stock solutions of prostaglandins were prepared in phosphate buffer (pH 7.4). Further dilutions were made in 0.9% sodium chloride. All other drugs were prepared in the appropriate bath fluid immediately before use. Statistical analysis: Levels of significance were calculated using Student's t-test.

RESULTS

Specificity of (---) INPEA-induced potentiation

The results obtained are summarized in Fig. 1 and Table 1. Oxytocin (25 $\mu\text{U/ml}$), PGE_1 (10 ng/ml), PGE_2 (10 ng/ml), $\text{PGF}_{2\alpha}$ (5 ng/ml), vasopressin (0.5 mU/ml), angiotensin

FIG. 1. Potentiating effect of (---) INPEA on the responses of isolated rat uterus to various stimulant drugs. Potentiation was compared over a dose which gave the minimal detectable response with each one of the agonist. Note the specificity of (---) INPEA-induced potentiation to oxytocin and prostaglandins as compared to other drugs. Figures in parentheses denote number of experiments with each agonist.

TABLE 1. Potentiating effect of (+) INPEA (1×10^{-5} g/ml) on the responses evoked by various stimulant drugs in isolated rat uterus.

Drug	Dose	Height of contraction (mm)		P Value
		Before (+) INPEA Mean $\pm$ S.E.	After (+) INPEA Mean $\pm$ S.E.	
Oxytocin (7)	25 uU/ml	13.5 $\pm$ 1.9	52 $\pm$ 4.5	<0.01
PGE ₁ (7)	10 ng/ml	7.1 $\pm$ 0.9	46 $\pm$ 2.3	<0.01
PGE ₂ (7)	10 ng/ml	4.9 $\pm$ 0.0	46.1 $\pm$ 4.3	<0.01
PGF _{2α} (7)	5 ng/ml	10.0 $\pm$ 1.3	38.8 $\pm$ 5.5	<0.01
Vasopressin (6)	0.5 mU/ml	3.8 $\pm$ 1.0	5.8 $\pm$ 1.3	>0.05
Angiotensin (5)	0.2 ng/ml	13.6 $\pm$ 2.7	20.2 $\pm$ 3.7	>0.5
5-HT (5)	5 ng/ml	13.2 $\pm$ 1.8	21 $\pm$ 3.3	>0.05
Bradykinin (5)	0.1 ng/ml	13.8 $\pm$ 2.2	20 $\pm$ 2.7	>0.5

Figures in parentheses denote the number of observations in each group.

(0.2 ng/ml), 5-HT (5 ng/ml) and bradykinin (0.1 ng/ml) produced reproducible contractions of the rat uterus. PGF_{2 α} was more potent on the rat uterus as the threshold dose required to produce a minimal detectable response was only one-half that of PGE₁ or PGE₂. Addition of (+) INPEA (1×10^{-5} g/ml) to the organ bath, per se, elicited no response whatsoever. The amplitude of oxytocin and prostaglandins (PGE₁, PGE₂ and PGF_{2 α})-evoked responses was, however, significantly increased ($P < 0.01$). The potentiation was more marked in the case of PGE₁ and PGE₂, as compared to PGF_{2 α} . The increase in the amplitude was about 4 fold in the case of oxytocin and PGF_{2 α} , while for the PGE₁ and PGE₂, it was 7 and 10 fold respectively. Although (:) INPEA potentiated the responses of vasopressin, angiotensin, 5-HT and bradykinin, the changes were not statistically significant ($P > 0.05$).

The mean ED₅₀ values of these drugs obtained from the log-dose response curves, before and after (+) INPEA are presented in Table II. After (+) INPEA, a marked reduction in the ED₅₀ values was noticed with oxytocin and prostaglandins as compared to other agents. The reduction in the ED₅₀ values was more pronounced for PGE₁ and PGE₂ as compared to PGF_{2 α} and oxytocin.

FIG. 2. Log-dose response curves of oxytocin before and after (+) INPEA on isolated strip of lactating rat mammary gland. Each point is the mean ($\pm$ S.E.) of five observations. Note the shift to the right of the dose-response curve after (+) INPEA.

Selectivity of (+) INPEA-induced potentiation

Isolated strip of rat mammary gland: Strips of rat mammary gland responded to doses of oxytocin down to 20 uU/ml in the bath fluid. The log-dose response relationship obtained was linear. Addition of (−) INPEA (1×10^{-5} g/ml) produced a weak inhibitory effect on the responses evoked by oxytocin and produced a slight shift to the right of the dose-response curve (Fig. 2). The results were not statistically significant ($P > 0.05$).

Isolated stomach (fundus) strip of rat: Sensitivity of the rat stomach strip was very high to PGE_1 (0.1 ng/ml) and the responses obtained were dose related. There was a slight shift to the left of the log-dose response curve of PGE_1 after the addition of (−) INPEA (1×10^{-5} g/ml) to the organ bath (Fig. 3). The apparent potentiation was, however, not significant ($P > 0.05$).

FIG. 3. Log-dose response curves of PGE_1 before and after INPEA on isolated rat stomach (fundus) strip. Each point is the mean ($\pm$ S.E.) of five observations. Note that there was a slight shift to the left of the dose-response curve after (+) INPEA.

FIG. 4. Log-dose response curves of PGE_2 before and after (−) INPEA on isolated guinea-pig ileum preparation. Each point is the mean ($\pm$ S.E.) of six experiments. Note the shift to the right of the dose response curve showing inhibition after (+) INPEA. The maximal response could not be obtained.

Isolated guinea-pig ileum: Guinea-pig ileum responded to doses of PGE_2 from 5 ng/ml onwards and maximal response could be obtained with graded doses. No tachyphylaxis was observed. Addition of (−) INPEA (1×10^{-5} g/ml) to the organ bath significantly ($P < 0.01$) inhibited the responses of PGE_2 . The log-dose response curve was shifted to the right in the presence of (−) INPEA in the bath fluid (Fig. 4).

Isolated guinea-pig tracheal chain: Dose related responses were obtained on the

FIG. 5. Dose-response curves to graded doses of $\text{PGF}_{2\alpha}$ before and after (+) INPEA on isolated guinea-pig tracheal chain. Each point is the mean ($\pm$ S.E.) of six experiments. Note the slight shift to the right of the dose-response curve after (+) INPEA showing a weak inhibitory effect.

smooth muscle of guinea-pig tracheal chain with graded doses of $\text{PGF}_{2\alpha}$. Addition of (+) INPEA (1×10^{-5} g/ml) to the bath fluid produced a weak inhibitory effect on the responses evoked by PGF_2 and showed a shift to the right of the dose-response curve. The changes observed were, however, statistically not significant ($P > 0.05$).

DISCUSSION

The results obtained in the present study reveal certain differences in the sensitivity patterns of rat uterus to various agonists. Among the prostaglandins, rat uterus was rather more sensitive to $\text{PGF}_{2\alpha}$ than PGE_1 . Similar observations have been made by Horton and Main (12) and Pickles et al. (13). (+) INPEA (1×10^{-5} g/ml) potentiated the responses of various drugs used in the present study. The potentiation caused by (+) INPEA appears to be selective for oxytocin and prostaglandins as is evident from the reduction of the ED50 values (Table II). The reduction in the ED50 values was more significant for oxytocin and prostaglandins. The order of potentiation was E_1 , E_2 , $F_{2\alpha}$, oxytocin, vasopressin = bradykinin, angiotensin, 5-HT (Table II).

TABLE 2. ED50 values of various stimulant drugs on isolated rat uterus before and after (+) INPEA.

Drug	ED50 Value		Value of ED50 Decreased by a factor of
	Control	After (+) INPEA (1×10^{-5} g/ml)	
Oxytocin	1.5×10^{-11}	3.75×10^{-11}	4.4
PGE_1	3.8×10^{-7}	3.36×10^{-8}	11.3
PGE_2	4.48×10^{-7}	3.08×10^{-8}	14.5
$\text{PGF}_{2\alpha}$	6.6×10^{-8}	1.13×10^{-8}	5.8
Vasopressin	8.2×10^{-9}	3.02×10^{-9}	2.7
Angiotensin	3.92×10^{-9}	1.88×10^{-9}	2.0
5-HT	7.6×10^{-8}	4.25×10^{-8}	1.8
Bradykinin	0.9×10^{-9}	0.33×10^{-9}	2.7

(+) INPEA-induced potentiation appears to be selective for the uterine muscle. Studies made with ^3H -oxytocin revealed that the receptor sites were different for oxytocin and prostaglandins in the uterine muscle (14). In the present study, (+) INPEA potentiated both oxytocin and prostaglandins-evoked responses on this tissue. (±) INPEA did not potentiate the oxytocin-evoked responses of isolated rat mammary strip. The reason for (±) INPEA not being able to potentiate the response of mammary gland to oxytocin could be that the receptors may be present within the cell and not on the membrane and the drug is somehow not able to reach the receptor site.

Similarly $\text{PGF}_{2\alpha}$ -evoked responses of gut and tracheal smooth muscle were not potentiated by (+) INPEA. This shows that the receptors present in these tissues may be different and it has been suggested that these are unlikely to exist as a single receptor, in view of the differences between prostaglandins and the multiplicity of their actions on different systems (15). On intestinal and bronchial smooth muscles, (±) INPEA exhibited a considerable inhibitory effect on prostaglandin-evoked responses. A similar inhibitory effect was also noticed on these tissues to the contractile effects of histamine and acetylcholine after (±) INPEA (unpublished observations) indicating a non-specific action.

Murmann (personal communication) indicated that (±) INPEA did not produce toxic effects on acute tests on mice and rats, and in chronic studies rats, dosed orally for 180 days. The drug also appears to be well tolerated in human subjects. A single oral dose (700 mg) and a single intravenous dose (75 mg) did not produce any untoward effect in hypertensive patients (16). However, pharmacological studies in human subjects are very few since (+) INPEA lacks practically any important blocking activity on beta adrenoceptors. The therapeutic results were not encouraging and clinical studies were not pursued.

Many side-effects have been observed with the clinical use of oxytocin and prostaglandins when used for induction of labour or therapeutic abortion. Severe water intoxication has been reported when oxytocin was used to terminate pregnancy (17–20). The use of prostaglandins for induction of labour and abortion has also been associated with undesirable side-effects, viz. nausea, vomiting, diarrhoea, fever, phlebitis and bronchoconstriction (21–22). At high dose levels, with prostaglandins in particular, the side-effects are distressing, while, at the lower levels, failures and incomplete abortions necessitating surgical intervention increase significantly.

It is evident from the present study that (±) INPEA more or less specifically potentiated oxytocin and prostaglandins-evoked responses of the uterine muscle. In view of this and its relative safety for clinical use, a combination of (±) INPEA with either oxytocin or prostaglandins or even with both may permit the use of smaller doses of oxytocin and prostaglandin when used for induction of labour or therapeutic abortion, thereby, limiting their side-effects and at the same time increasing their therapeutic efficacy.

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