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Effects of Propiverine and Its Metabolite Propiverine-N-Oxide on Bladder Contraction and Salivation in Mini Pigs

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Key Words
Propiverine · Propiverine N-oxide · Mini pig · Detrusor · Salivation · Anticholinergics · Metabolite

Abstract
Purpose: The objective of this study was to evaluate the influence of propiverine-HCl (P4) and propiverine-N-oxide (P4NO), one of the major metabolites of P4, on bladder contraction in a standardized in vivo model. Additionally, salivary flow measurements enabled the evaluation of hyposalivation, one of the most predominant anticholinergic side effects. ***Materials and Methods:*** Ten male mini pigs were anesthetized. P4 (0.4 mg/kg b.w.) and P4NO (0.422 mg/kg b.w.) were administered intravenously. Bladder contractions were induced through sacral anterior root stimulation and cystometrogram evaluation was performed. For stimulation-induced salivary flow measurements, the lingual nerve was exposed for neurostimulation. The effects of P4 and P4NO on stimulation-induced bladder contraction and salivation were evaluated in 5 mini pigs, respectively. ***Results:*** In all experiments, for each animal reproducible intravesical pressure values (Pves) were elicited during sacral anterior root stimulation before administration of the study drug. After administration of P4, Pves decreased by 64% whereas P4NO decreased Pves by 28%. Inhibition of salivary flow with P4 and P4NO was 71 and 32%, respectively. Directly following intravenous administration of P4, a short-term and reversible period of mild fluctuations in heart rate was observed. Administration of P4NO revealed no changes in either heart rate, or blood pressure. ***Conclusion:*** All of the investigated parameters revealed less anticholinergic effects for P4NO compared to P4. Under the experimental conditions described above, it may be assumed that P4NO behaves as a substance with poor anticholinergic effects with respect to side effects. As expected, P4 showed anticholinergic effects on bladder contraction and salivation.

Introduction

In urology, substances with anticholinergic properties are administered in the treatment of all types of bladder overactivity. The therapeutic effect is based on competitive inhibition of the postganglion and intramural muscarinic receptors of the urinary bladder. This causes the natural neurotransmitter acetylcholine to assert less influence, thus suppressing bladder contraction.

Anticholinergic therapy is the first-line treatment option for patients with overactive bladder. Different drugs are available (e.g. darifenacin [1], oxybutynin [2], propiverine (P4) [3], solifenacin [4], tolterodine [5], trospium chloride [6]).

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Oxybutynin has both an antimuscarinic and a direct muscle-relaxant effect. P4 is a smooth-muscle-relaxing agent with anticholinergic and calcium-antagonistic properties demonstrated in in vivo and in vitro studies. Darifenacin, solifenacin, tolterodine and trospium chloride are pure antimuscarinics. Although clinical efficacy of some of these drugs has been proven to be high, its use is limited by antimuscarinic adverse effects, possibly related to active metabolites.

Most anticholinergic drugs metabolize in the liver into more or less active metabolites. The extent to which the active metabolites contribute to pharmacological effects at the target tissue depends on their affinity to the receptors. In the case of some anticholinergic drugs, active metabolites might contribute to the overall clinical profile of the drug. Tolterodine and oxybutynin, for example, are extensively metabolized in the liver to the active metabolites 5-hydroxymethyl-tolterodine and N-desethyloxybutynin, respectively [7]. P4 is also metabolized in the liver and propiverine-N-oxide (P4NO) is the principal metabolite in human blood [8]. Although the effects of P4 metabolites have extensively been studied in vitro [9, 10], sparse information of in vivo action of P4NO is available.

The objective of this study was to evaluate the influence of P4 and P4NO on electrostimulation-induced bladder contraction in a standardized mini pig model [11]. Additionally, salivary flow measurements enabled the evaluation of hyposalivation, one of the most predominant anticholinergic side effects.

Materials and Methods

Ten adult male Göttinger mini pigs (b.w. 25–37 kg, Ellegaard Mini Pigs ApS, Denmark) were anesthetized by i.m. administration of 200 mg ketamine (Intervet, Unterschleißheim, Germany) and 15 mg midazolam. The animal was intubated and connected to an artificial respirator and anesthesia was maintained with 2.3% isoflurane (Abbott, Wiesbaden, Germany) and an oxygen supply of 40%. Moreover, 0.3 mg/kg/h piritramide (Janssen Cilag, Neuss, Germany) was administered continuously intravenously via an infusion pump. The carotid artery was cannulated for blood pressure control and the jugular vein for perioperative infusion with Ringer's solution and administration of the study drugs (P4 and P4NO; Apogepha GmbH, Dresden, Germany). The lingual nerves were exposed and a cuff electrode was placed around the nerve and connected to an external neurostimulator.

The urinary bladder was exposed by a mid-abdominal incision and a catheter was inserted into the bladder via a cystostomy for cystometrographic measurements with computerized urodynamic equipment (Wiest 8000, Unterhachingen, Germany). The bladder was filled with 200 ml physiological saline (approx. 2/3 of maximal bladder capacity). Both ureters were cannulated and bilateral ureterocutaneostomy was performed in order to maintain a constant bladder volume during the duration of the study. The posterior urethra was ligated in order to avoid urine leakage during sacral anterior root stimulation (SARS).

Laminectomy and identification of the sacral roots followed closure of the abdomen and funneling the connecting wires of the electrodes to the body surface. A modified Brindley electrode (Finetech, London, UK) was placed at the sacral anterior root S2 and connected to an external neurostimulator.

Individual electrostimulation of the lingual nerve and the sacral anterior root S2 with standardized stimulation parameters was carried out after completion of the operative procedures. In addition, stimulation-induced salivation of the submandibular gland was evaluated by a pad test. The pad was weighed before and after stimulation in order to assess the amount of salivation adsorbed during stimulation of the lingual nerve (3 min). A recovery period of at least 20 min elapsed between each neurostimulation to avoid neurostimulation-induced fatigue of the detrusor muscle and the submandibular glands. Blood pressure and heart rate (HR) were continuously monitored throughout the entire study. During the study, hypothermia was prevented by constant maintenance of the core body temperature with an electric blanket (37°C) and infusion with warmed Ringer's solution.

The effects of P4 (0.4 mg/kg b.w.) and P4NO (0.422 mg/kg b.w.) on stimulation-induced bladder contraction and salivation were evaluated in five mini pigs, respectively. The chosen dose of P4NO is molar equivalent to the dose of P4. Drugs were dissolved in 20 ml 0.9% physiological saline and were given intravenously within 60 s (jugular vein). 40 min after administration of the substance or after at least two consecutive neurostimulations, atropine (4 mg) was also administered intravenously following SARS and neurostimulation of the lingual nerve (fig. 1).

The following stimulation parameters were used for SARS: biphasic rectangular pulses, peak to peak amplitude 4 mA, duration of plateau phase 200 μs, stimulation frequency 20 Hz, total time of each stimulation 15 s.

The following stimulation parameters were used for stimulation of the lingual nerve: biphasic rectangular pulses, peak to peak amplitude 4 mA, duration of plateau phase 2 ms, stimulation frequency 5 Hz, total time of each stimulation 3 min.

The protocol was approved by the institutional animal care and use committee of the University Hospital Mannheim, Karl Ruprecht University Heidelberg, and was in line with EU guidelines. All values are expressed as the mean ± SEM. Statistical significance was determined by Student's t test with $p < 0.05$ considered statistically significant.

Results

Bladder Pressure

In all experiments, for each animal reproducible intravesical pressure values (Pves) were elicited during SARS before administration of the model drugs (fig. 2).

Forty minutes after i.v. administration of P4, the neurostimulation-induced rise in Pves dropped from

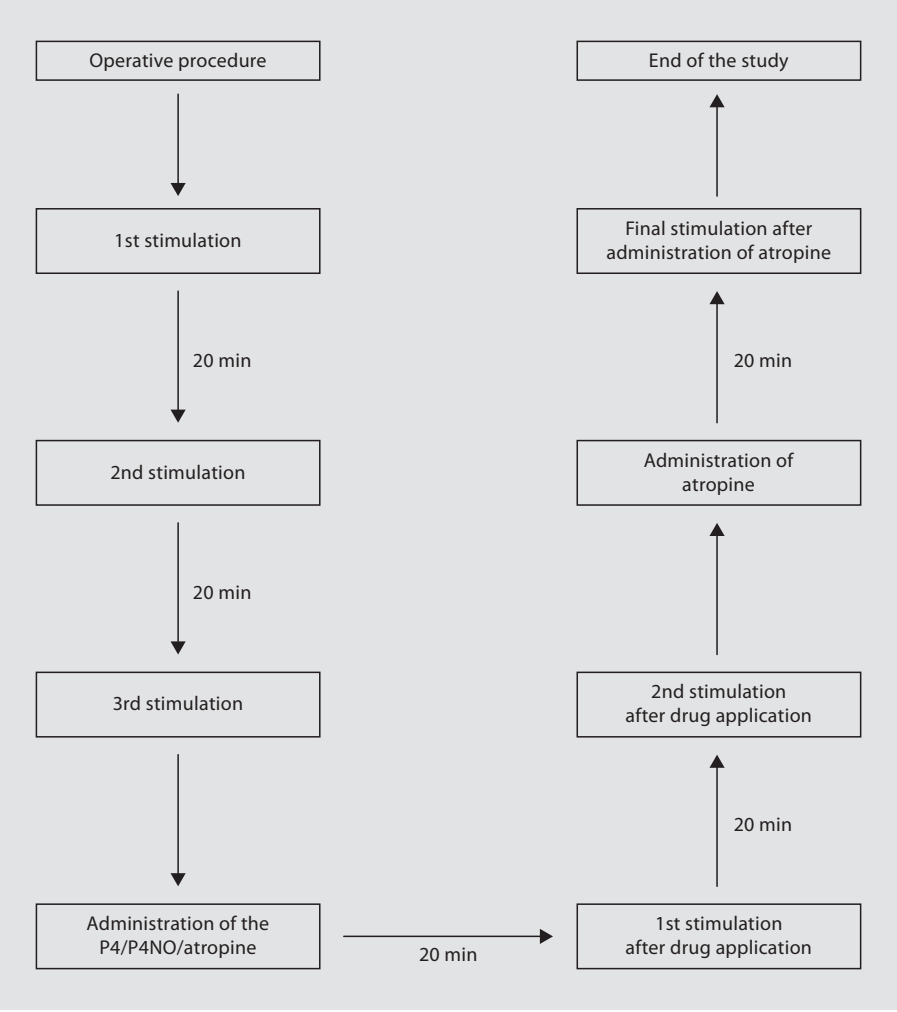

Fig. 1. Flow chart of the stimulation study.

42.0 cm H_2O before drug administration to 16.8 cm H_2O ($p = 0.056$). After additional administration of atropine, stimulation-induced Pves dropped further to 5.4 cm H_2O ($p < 0.05$).

Forty minutes after i.v. administration of P4NO, neurostimulation-induced rise in Pves dropped from 31.8 to 25.0 cm H_2O ($p = 0.077$). After additional administration of atropine, stimulation-induced Pves further decreased to 10.8 cm H_2O ($p < 0.05$) (table 1).

Salivation

Before administration of the study drug, stimulation-induced salivation was reproducible for each animal in all studies (table 2). In one experiment the electrodes were dislocated (P4NO) and in another one technical failure caused inadequate evaluation of salivary flow (P4NO).

After administration of P4, neurostimulation-induced salivary flow decreased from 109.4 mg/3 min to 50 mg/3 min ($p < 0.05$). Additional intravenous administration of atropine induced a further inhibition of salivary flow to 4.2 mg/3 min ($p < 0.05$).

Under the influence of P4NO, neurostimulation-induced salivary flow decreased from initial 53.3 mg/3 min to 39.3 mg/3 min ($p = 0.184$). After atropine, stimulation-induced salivary flow dropped further to 19.7 mg/3 min; however, this decrease was not significant ($p = 0.108$).

HR and Blood Pressure

During the operative procedures, all animals were stable in terms of blood pressure and HR (tables 3, 4). Directly following intravenous administration of P4, a significant increase in HR at a mean of 17% of the initial value was observed during the first 10 min ($p < 0.05$). The

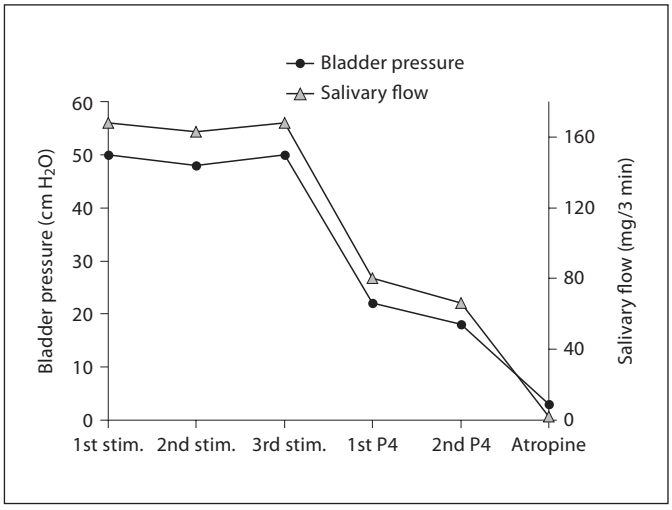

Fig. 2. Representative course of bladder pressure and salivary flow of one study with P4.

Table 1. Stimulation-induced bladder pressure

	Before cm H_2O	After cm H_2O	Atropine cm H_2O
P4			
No. 1	50 (100)	18 (36)	3 (6)
No. 2	62 (100)	6 (10)	4 (8)
No. 3	25 (100)	17 (68)	4 (16)
No. 4	37 (100)	10 (27)	4 (11)
No. 5	36 (100)	33 (92)	12 (24)
Mean ± SEM	42.0 ± 6.4	16.8 ± 4.6	5.4 ± 1.7
P4NO			
No. 1	47 (100)	45 (96)	20 (43)
No. 2	14 (100)	10 (71)	5 (36)
No. 3	22 (100)	16 (73)	7 (32)
No. 4	18 (100)	14 (78)	6 (33)
No. 5	58 (100)	40 (69)	16 (28)
Mean ± SEM	31.8 ± 8.7	25.0 ± 7.3	10.8 ± 3.0

Figures in parentheses are percentages.

HR returned to baseline value after 20 min. This increase in HR was not observed in the P4NO group.

After administration of the study drugs, there was no change in mean arterial pressure (MAP) compared with baseline: 61.8 versus 62.8 mm Hg for P4 and 64.6 versus 64.4 mm Hg for P4NO. MAP was calculated using the formula: MAP (mm Hg) = (systolic + 2 × diastolic)/3. Gas analysis throughout the study showed no relevant changes.

Table 2. Stimulation-induced salivary flow

	Before mg/3 min	After mg/3 min	Atropine mg/3 min
P4			
No. 1	168 (100)	66 (39)	2 (1)
No. 2	68 (100)	20 (29)	10 (15)
No. 3	160 (100)	110 (69)	5 (3)
No. 4	131 (100)	34 (26)	2 (2)
No. 5	20 (100)	20 (100)	2 (10)
Mean ± SEM	109.4 ± 28.4	50.0 ± 17.2	4.2 ± 1.6
P4NO			
No. 1	88 (100)	60 (68)	50 (57)
No. 2	60 (100)	54 (90)	8 (13)
No. 3	–	–	–
No. 4	–	–	–
No. 5	12 (100)	4 (33)	1 (8)
Mean ± SEM	53.3 ± 22.2	39.3 ± 17.0	19.7 ± 15.3

Figures in parentheses are percentages.

Discussion

In a porcine model, electrostimulation of the sacral roots responsible for bladder contraction leads to detrusor activation with consecutive rise in bladder pressure. This stimulation-induced rise in bladder pressure can be urodynamically evaluated and objectively quantified. Standardized stimulation parameters result in reproducible, stimulation-induced bladder contractions suitable for pharmacological evaluation of drug effects on bladder contraction [12]. In this study, the sacral root S2 was stimulated to evoke neurogenic-induced bladder contractions. Further expansion of the porcine study procedure makes the investigation of typical anticholinergic side effects such as hyposalivation also possible [11, 13].

In this experimental set-up, the study drugs were given intravenously. Therefore, first-pass metabolization in the liver and gut is negligible. This makes the presented model suitable for comparative investigations of a parent drug and its metabolites with respect to anticholinergic effects on the detrusor muscle and on salivary glands.

The therapeutic effect of P4 is due to inhibition of calcium influx into smooth muscle cells and competitive antagonism with acetylcholine at muscarinic receptor sites [3]. P4 undergoes extensive first-pass metabolism (N-ox-

Table 3. Heart rate

	Before min^{-1}	After min^{-1}	Atropine min^{-1}
P4			
No. 1	113 (100)	135 (119)	139 (123)
No. 2	102 (100)	120 (118)	129 (126)
No. 3	118 (100)	136 (115)	144 (122)
No. 4	87 (100)	104 (120)	124 (143)
No. 5	54 (100)	63 (117)	66 (122)
Mean ± SEM	94.8 ± 11.5	111.6 ± 13.4	120.4 ± 14.1
P4NO			
No. 1	67 (100)	67 (100)	66 (99)
No. 2	86 (100)	87 (101)	89 (103)
No. 3	110 (100)	104 (95)	110 (100)
No. 4	88 (100)	90 (102)	103 (117)
No. 5	66 (100)	67 (101)	67 (101)
Mean ± SEM	83.4 ± 8.0	83.0 ± 7.1	87.0 ± 9.0

Figures in parentheses are percentages.

Table 4. Mean arterial pressure

	Before mm Hg	After mm Hg	Atropine mm Hg
P4			
No. 1	47 (100)	48 (102)	51 (109)
No. 2	80 (100)	82 (102)	80 (100)
No. 3	67 (100)	68 (101)	54 (81)
No. 4	66 (100)	67 (101)	75 (136)
No. 5	49 (100)	49 (100)	53 (108)
Mean ± SEM	61.8 ± 6.2	62.8 ± 6.4	62.6 ± 6.2
P4NO			
No. 1	50 (100)	51 (102)	43 (86)
No. 2	80 (100)	80 (100)	81 (101)
No. 3	64 (100)	65 (102)	63 (98)
No. 4	90 (100)	88 (98)	78 (87)
No. 5	39 (100)	38 (97)	40 (103)
Mean ± SEM	64.6 ± 9.4	64.4 ± 9.2	61.0 ± 8.6

Figures in parentheses are percentages.

idation, O- and/or N-dealkylation). Only 3% of the drug is recovered unchanged in the urine and bile after oral administration. The metabolite P4NO can be recovered from the human urine for 20% of the original dose [8].

In vivo effects of anticholinergic drugs depend on receptor affinity and on free plasma concentration. Recent work has shown that P4NO has lower affinity for muscarinic receptors than P4 [14]. These data obtained from in vitro studies were in accordance with the results of our functional in vivo study. Furthermore, it is known that the free plasma concentration is higher for P4NO than for P4 [15], and therefore anticholinergic effects should be less pronounced compared to P4.

The results of the present study demonstrate that the principal metabolite of P4, P4NO, possesses less anticholinergic properties. With respect to the inhibition of stimulation-induced bladder contraction, the effect of P4NO was maximal 30%, whereas maximal inhibition of P4 was 90%. Salivation flow evaluation revealed similar results, although in the group with P4NO, two measurements failed due to technical problems. The variations in initial stimulation-induced Pves and salivation might be due to interindividual variations of the nervous and/or urinary system in each animal. Nevertheless, all investigated parameters were reproducible in each animal before the test substance was administered.

P4 failed to inhibit stimulation-induced Pves and salivary flow in one pig. On the other hand, the HR of these animals did react appropriately after application of the study drug. The reasons for this are unclear. An explanation could be that the lower urinary tract of these animals was insensitive to P4 since additionally administration of atropine inhibited Pves and salivation adequately.

Contrary to P4 and atropine, no relevant changes in HR or blood pressure were observed after administration of P4NO. A temporary rise in HR was observed immediately after intravenous administration of P4 and atropine. However, this returned to normal within 25 min.

Recently, in vitro studies suggested that the metabolite M5 (the N-oxide of propiverine) reduces detrusor contractions elicited by electric field stimulation [9]. This seems to be in contrast to our results and might be explained with differences in experimental setup. The results of the above-mentioned study are derived from in vitro studies on urothelium-denuded detrusor strips. Furthermore, we used a single dose and a weaker inhibition might reflect this. Therefore, comparison of the data is difficult due to experimental differences. Furthermore, the concentration used in in vitro studies cannot be transferred to an in vivo situation.

Anticholinergic drug therapy typically does not suppress detrusor overactivity but mainly increases bladder capacity, compliance and volume of first unstable contraction [16]. Furthermore, urodynamic studies in humans cannot unequivocally demonstrate a decrease in maximum bladder pressure. However, some groups re-

ported a change in maximum bladder pressure due to anticholinergics [17, 18]. In these studies, the maximum detrusor pressure was lowered significantly in the P4 group from 56.8 to 37.8 cm H_2O and 43.8 to 27.1 cm H_2O, respectively. Moreover, the latter studies reported a suppression of phasic detrusor overactivity by 63%. The bladder contraction evoked in our study is elicited by stimulating the sacral micturition center and therefore is comparable with maximum bladder contraction. In our study, maximum bladder contraction was suppressed by P4 by 60%.

In the presented animal model, only the efferent motor nerves were activated to induce bladder contraction, while the afferent pathway, which is believed to be involved in the pathogenesis of detrusor overactivity, was excluded. Recent studies showed that several anticholinergics might modulate bladder function through afferent nerve fibers [19, 20], and it cannot be excluded that P4NO in a chronic animal model might influence bladder function. However, an acute inhibitory effect of P4NO on efferent detrusor activation could not be demonstrated in this study.

In summary, all of the investigated parameters revealed less anticholinergic effects for P4NO compared to P4. Under the experimental conditions described above, it may be assumed that P4NO behaves as a substance with poor anticholinergic effects with respect to side effects. As expected, P4 showed anticholinergic effects on bladder contraction and salivation.

This study provides information about in vivo efficacy of P4 and its principal metabolite P4NO and is probably more elucidating and representative than investigation of strips.

Acknowledgments

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