

## INTERACTIVE EFFECTS OF DIFFERENT DURATION OF LITHIUM PRETREATMENT WITH AMIKACIN AND GENTAMICIN ON APOMORPHINE-INDUCED LICKING IN RATS

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### ABSTRACT

In this study the hypothesis that aminoglycoside antibiotics and lithium may influence apomorphine-induced licking via their effects on phosphoinositide pathways and calcium stores were investigated in male rats. Subcutaneous administration of apomorphine (0.1, 0.25 and 0.5 mg/kg) to rats induced licking in a dose-dependent manner and the maximum response was obtained by the dose of 0.5 mg/kg of the drug. Intracerebroventricular injections of amikacin (5, 25 and 50  $\mu\text{g}/\text{rat}$ ) and gentamicin (10, 20 and 40  $\mu\text{g}/\text{rat}$ ) decreased the apomorphine-induced licking significantly. Pretreatment of animals with lithium (600 mg/l) for 7, 14 and 21 days increased licking induced by apomorphine. The inhibitory effects of amikacin and high dose of gentamicin were not affected by lithium pretreatment for 14 and 28 days. These findings indicate the possible involvement of phosphoinositide cascade in alterations of apomorphine-induced licking by aminoglycoside antibiotics and lithium in the brain. Also it is suggested that type and dose of aminoglycoside antibiotics and duration of lithium administration probably have different effects on responses mediated by phosphoinositide hydrolysis.

**Key words:** Apomorphine, Licking, Amikacin, Gentamicin, Phosphoinositides

### INTRODUCTION

Dopamine receptors have been separated into two functionally distinct receptors, D1 stimulating dopamine sensitive adenylyl cyclase and D2 inhibiting cyclase or unassociated with enzyme (1,2). The phosphoinositide intracellular signaling pathway, which triggers calcium release from intracellular stores has also been proposed to link to D1 and D2 dopamine receptors in both brain and peripheral tissue (3-5). Involvement of dopaminergic system in licking behavior is well established (6). Licking is a stereotyped behavior which is more closely associated with the caudate striatum dopaminergic pathways (7) and can be used as a model for understanding of various nervous system functions. Both D1 and D2 dopamine receptors activation are required to induce licking. (6). Apomorphine is a nonselective D1 and D2 dopamine receptor agonist (8-10). It has been shown that aminoglycoside antibiotics prevent the action of cellular phospholipases and

interact with anionic phospholipids such as phosphoinositides (11). This may influence the inhibition of inositol triphosphate (IP3) and diacylglycerol (DAG) formation, the second messengers of the phosphoinositide cascade (12). Lithium is widely accepted as the drug of first choice in the treatment of manic and prophylaxis of bipolar affective disorders (13,14). Despite of vast amount of investigations in this biochemical phenomena no study has explained adequately the mechanism of the clinical action of lithium. However, there are increasing evidences that lithium exerts its therapeutic action by interfering with phosphoinositide metabolism and preventing recycling of inositol by an uncompetitive inhibition of inositol monophosphatase (15).

Our previous works have shown that lithium can alter responses that are mediated by phosphoinositides pathway (16-18,19). The present study was designed to clarify the interactive effects of aminoglycoside

antibiotics with lithium on dopaminergic mechanisms as it was tested by the licking model.

## MATERIALS AND METHODS

**Animals:** Male albino rats (150-250 g) were housed in group of 5 in cages in a controlled temperature room with a 12 h light-dark cycle. Food and water were freely available except during the experiments.

**Licking measurement:** Animals were placed individually in a glass cylinder (25 cm wide, 25 cm long) and a mirror was arranged in an oblique position under the cylinder to make recording of licking possible. Animals were allowed to habituate for 30 min before drugs administration. Immediately after drug administration, they were placed into the cylinder and the number of licks (protrusion of the tongue against the cylinder wall or floor) were recorded by a directed observation during a 60 min period.

**Drugs:** The following drugs were used: apomorphine HCl (Macfarlan Smith Ltd), lithium chloride (Merck, Germany), pentobarbital (Park-Davis, France), amikacin and gentamicin (Pharmagreen, UK). All drugs were dissolved in sterile water. The drugs were prepared immediately before the use and were injected in a volumes of 2 ml/kg for subcutaneous injection and 5 ml/kg for intraperitoneal injection and in a volume of 2  $\mu$ l /rat for intracerebroventricular injections.

**Chronic guide cannulae implantation:** For intracerebroventricular injection the rats were anaesthetized with pentobarbital (50 mg/kg) and were placed in a stereotaxic frame (David Kopf Instruments, USA) and a stainless steel guide cannulae was implanted in the left lateral ventricle at the following coordinates: AP=0.8 mm, L=1.6 mm (both with respect to bregma) and V = 3.5 from the dura. For the recovery period, animals were kept individually for 7 days. The drugs were injected in a volume of 2  $\mu$ l over a period of 2 min, by means of an internal cannulae (27-gauge) connected by polyethylene tubing to a 10- $\mu$ l Hamilton syringe and the injection cannulae was left in place for 1 min further before it was being slowly withdrawn.

**Lithium experiments:** For lithium administration, LiCl at 600 mg/l was dissolved in drinking water

and was fed to rats for periods of 7, 14, 21 and 28 days. Control rats received tap water.

**Determination of serum lithium levels:** Lithium measurements were made after 14 and 28 days of chronic lithium treatment. Animals were sacrificed by decapitation, and blood was collected from the neck wound into siliconized tubes. Serum was immediately separated by micro-centrifuge (Eppendorf, Germany) and diluted in distilled and deionized water for estimation of lithium. Lithium levels in serum samples were then determined using atomic absorption spectrophotometer (Shimadzu, AA 670 Japan). Reading was made in triplicate at a wavelength of 670.8 nm, and peak height measurements were compared with values of standards of the known concentration made up in diluted serum.

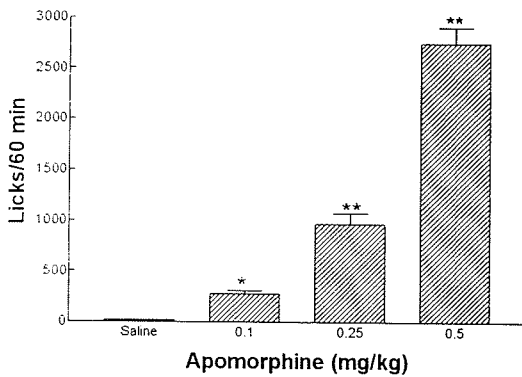
**Statistical analysis:** ANOVA followed by Newman Keuls tests were used to evaluate the significance of the results. Differences with  $p < 0.05$  were considered statistically significant.

## RESULTS

**Effect of apomorphine on licking behavior in rats:** Subcutaneous injection of different doses of apomorphine (0.1, 0.25, 0.5 mg/kg) induced licking in a dose-dependent manner. The maximum response was achieved with concentration of 0.5 mg/kg of the drug (Fig. 1).

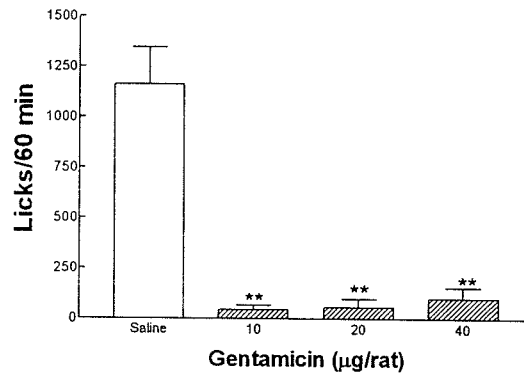
**Effects of amikacin and gentamicin on licking induced by apomorphine:** Intracerebroventricular injection of different doses of amikacin (5, 25 and 50  $\mu$ g/rat) decreased the licking response induced by apomorphine (0.25 mg/kg, s.c.) (Fig. 2). Intracerebroventricular administration of different doses of gentamicin (10, 20 and 40  $\mu$ g/rat) also decreased apomorphine (0.25 mg/kg, s.c.) response significantly (Fig. 3). The maximum inhibitory effects of amikacin and gentamicin on apomorphine response were achieved by intracerebroventricular administration of the drug in the amounts of 50  $\mu$ g/rat and 10  $\mu$ g/rat respectively.

**Effects of lithium pretreatment on apomorphine-induced licking:** Administration of lithium (600mg/l) in drinking water for 7, 14 and 21 days increased the number of licking counts induced by apomorphine (0.25 mg/kg, s.c.) significantly. The maximum effect of lithium on apomorphine



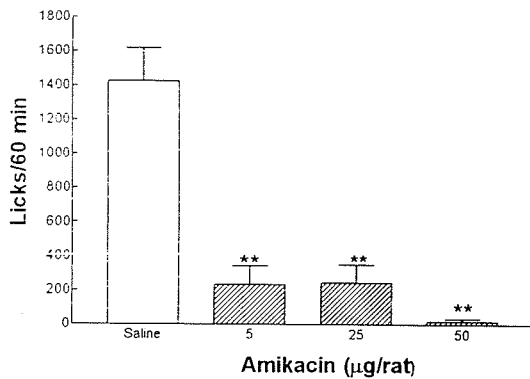
**Fig 1.** Effect of different doses of apomorphine on licking in rats.

Animals were injected subcutaneously either with saline (2ml/kg) or apomorphine (0.1, 0.25 and 0.5 mg/kg). Licking was recorded during 60 min after apomorphine injection. Each point is the mean  $\pm$ SEM of 7 animals. \*\* $P < 0.01$  and \* $P < 0.05$  different from saline control group.



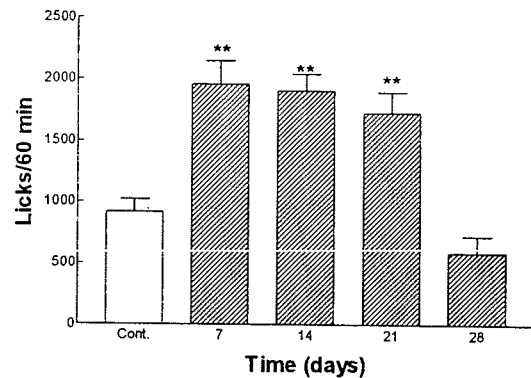
**Fig 3.** Effects of gentamicin on licking induced by apomorphine.

Animals were injected intracerebroventricularly with saline (2  $\mu$ l/rat) or gentamicin (10, 20 and 40  $\mu$ g/rat). Licking was recorded during 60 min. after apomorphine (0.25 mg/kg, S.C.) injection. Each point is the mean  $\pm$ SEM of 7 animals. \*\* $P < 0.01$  different from saline control group.



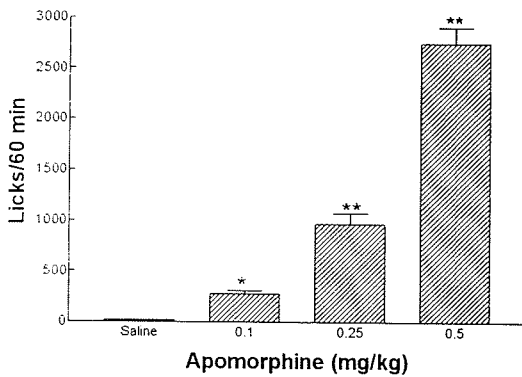
**Fig 2.** Effects of amikacin on licking induced by apomorphine.

Animals were injected intracerebroventricularly either with saline (2  $\mu$ l/rat) or amikacin (5, 25 and 50  $\mu$ g/rat). Licking was recorded during 60 min after apomorphine (0.25 mg/kg, S.C.) injection. Each point is the mean  $\pm$  S.E.M. of 7 animals. \*\* $P < 0.01$  different from saline control group.



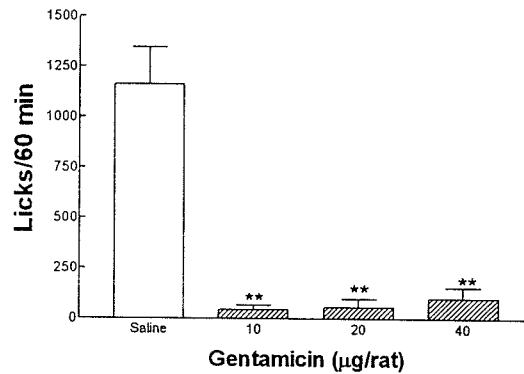
**Fig 4.** Effects of lithium pretreatment on apomorphine-induced licking.

Chronic lithium pretreated rats received LiCl 600 mg/l in drinking water for a 7, 14, 21 and 28 days periods. Control rats received tap water. Animals were injected subcutaneously either with saline (2 ml/kg) or apomorphine (0.25 mg/kg). Licking was recorded during 60 min. after apomorphine injection. Each point is the mean  $\pm$ SEM of 7 animals. \*\* $P < 0.01$  different from control group.



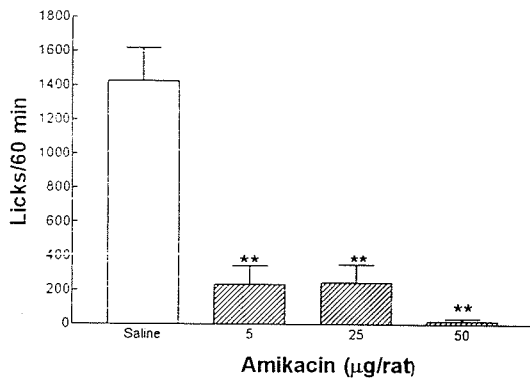
**Fig 1.** Effect of different doses of apomorphine on licking in rats.

Animals were injected subcutaneously either with saline (2ml/kg) or apomorphine (0.1, 0.25 and 0.5 mg/kg). Licking was recorded during 60 min after apomorphine injection. Each point is the mean  $\pm$ SEM of 7 animals. \*\* $P < 0.01$  and \* $P < 0.05$  different from saline control group.



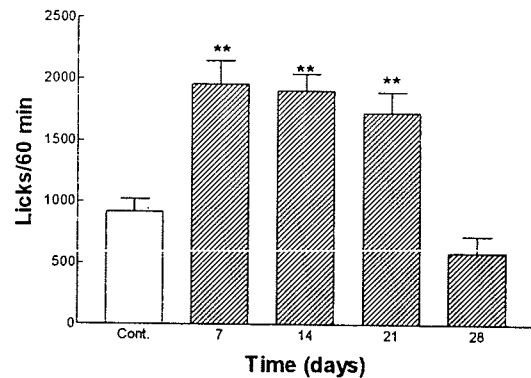
**Fig 3.** Effects of gentamicin on licking induced by apomorphine.

Animals were injected intracerebroventricularly with saline (2  $\mu$ l/rat) or gentamicin (10, 20 and 40  $\mu$ g/rat). Licking was recorded during 60 min. after apomorphine (0.25 mg/kg, S.C.) injection. Each point is the mean  $\pm$ SEM of 7 animals. \*\* $P < 0.01$  different from saline control group.



**Fig 2.** Effects of amikacin on licking induced by apomorphine.

Animals were injected intracerebroventricularly either with saline (2  $\mu$ l/rat) or amikacin (5, 25 and 50  $\mu$ g/rat). Licking was recorded during 60 min after apomorphine (0.25 mg/kg, S.C.) injection. Each point is the mean  $\pm$  S.E.M. of 7 animals. \*\* $P < 0.01$  different from saline control group.



**Fig 4.** Effects of lithium pretreatment on apomorphine-induced licking.

Chronic lithium pretreated rats received LiCl 600 mg/l in drinking water for a 7, 14, 21 and 28 days periods. Control rats received tap water. Animals were injected subcutaneously either with saline (2 ml/kg) or apomorphine (0.25 mg/kg). Licking was recorded during 60 min. after apomorphine injection. Each point is the mean  $\pm$ SEM of 7 animals. \*\* $P < 0.01$  different from control group.

response was obtained after 7 days. Pretreatment of animals with lithium for 28 days did not elicit any significant alteration on the apomorphine-induced licking in comparison with control group (Fig. 4).

**Effects of amikacin and gentamicin on apomorphine-induced licking in animals pretreated with lithium for 14 and 28 days:**

Pretreatment of animals with lithium (600 mg/l in drinking water) for 14 and 28 days did not affect the inhibitory responses of different doses of amikacin (5, 25 and 50 µg/rat, i.c.v.) and gentamicin (40 µg/rat, i.c.v.) on licking induced by apomorphine (0.25 mg/kg, s.c.). However the inhibitory effect of gentamicin (10, 20 µg/rat, i.c.v.) decreased in 28 days lithium treated animals. Significant difference on inhibitory response of gentamicin (20 µg/rat, i.c.v.) was observed between animals received lithium for 14 and 28 days. Different administration periods of lithium (14 and 28 days) also changed the licking induced by apomorphine (0.25 mg/kg, s.c.) in saline treated group (Tables 1 and 2).

**Serum lithium concentration:** In the experiment, the total lithium serum concentrations after 14 and 28 days were  $9.85 \pm 3.23$  and  $14.42 \pm 3.43$  µg/ml respectively (mean  $\pm$  SEM, n=10).

## DISCUSSION

The present data indicate that subcutaneous injection of dopamine receptor agonist apomorphine (8,10) induced licking response which is in agreement with our previous work (6). Since both D1 and D2 dopamine receptors activation are required to induce licking (6), it seems that apomorphine via interactions with D1 and D2 dopamine receptors caused this behavior. One of the major finding of the present study is that intracerebroventricular administration of amikacin and gentamicin as aminoglycoside antibiotics reduced the number of licking induced by apomorphine, indicating the possible interactions of dopaminergic mechanisms with aminoglycoside antibiotics in central nervous system.

Accumulated evidence clearly suggested that dopaminergic activation of phospholipase C and stimulation of inositol phosphate formation in the brain is mediated by a D1 dopamine receptor subtype which is distinct from the D1 receptor

that activates adenylyl cyclase (3). There is also evidence that in certain cells, D2 stimulation may activate phospholipase C that hydrolyses the membrane phospholipid phosphatidyl inositol bisphosphate (PIP2) which increases inositol triphosphate (IP3) and intracellular calcium (4,5). Since aminoglycoside antibiotics can prevent the action of cellular phospholipases and inhibit the formation of inositol triphosphate (IP3) and diacylglycerol (DAG) which are the second messengers of phosphoinositide cascade (11, 12), the inhibitory effects of amikacin and gentamicin on apomorphine response may be related to their interactions with phosphoinositide metabolism. In addition recent studies have shown that aminoglycoside antibiotics act as N-type calcium channel blockers in the central nervous system (20-22). Furthermore, aminoglycoside antibiotics are polybasic and highly hydrophilic compounds which interfere with the binding of calcium to phospholipid monolayers and biomembranes (23) and reduce calcium uptake into synaptosome (21,24). However the capacity of amikacin and gentamicin to block N-type calcium channel and to decrease neuronal calcium availability is another possibility. Interactions between aminoglycoside antibiotics and phosphoinositide cascade were shown in our previous works (18). Lithium salts remain one of the most widely used treatment for depressive illness probably by reduction in free inositide. Another major finding of the present investigation is that lithium pretreatment can increase apomorphine-induced licking after 7,14 and 21 days and the maximum effects was observed in the first week of the treatment. Lithium alone did not produced licking. Since lithium also exerts a profound alteration in phosphoinositide cascade by inhibiting the inositol-1-phosphatase (25, 26), the effects of 7, 14 and 21 days lithium treatment on licking response could be accounted for, at least partially by a lithium-induced accumulation of IP3 and the increase of the intracellular calcium release. It is also suggested that duration of lithium treatment may be involved in alterations of apomorphine-induced licking by perturbation with phosphoinositide metabolism in the brain. Our previous works have shown that lithium can affect the responses mediated by the second

messengers of the phosphoinositide pathway (15-17). The inhibitory effects of amikacin and high dose of gentamicin on apomorphine response was not blocked in animals pretreated with lithium for 14 and 28 days. It seems that the inhibitory effects of aminoglycoside antibiotics are higher than that to be affected by lithium in different administration periods. Also it is suggested that type and dose of aminoglycoside antibiotics probably have different effects on phosphoino-

side metabolism. In conclusion our results show a possible involvement of phosphoinositide pathway in alterations of licking response induced by apomorphine. There are, however, other mechanisms for aminoglycosides and lithium and interactions between two agents need not to occur by a common pathway of the phosphoinositide cascade. Further experiments are required to clarify these points.

Table 1. Effects of different doses of amikacin on apomorphine-induced licking in animals pretreated with lithium for 14 and 28 days

Time (days)	Licks/60 min			
	Saline (2 µl/rat)	Amikacin (5 µg/rat)	Amikacin (25 µg/rat)	Amikacin (50 µg/rat)
Control	1427±190	232±180	245±104	19±14
Li 14 days	1754±196 <sup>a</sup>	38±27	212±45	128±118
Li 28 days	529±133**	33±19	574±192	213±19

Animals were injected either with saline (2 µl/rat, i.c.v.) or amikacin (5, 25 and 50 µg/rat, i.c.v.) immediately before apomorphine (0.25 mg/kg, s.c.). Licking was recorded during 60 min. after apomorphine injection. Each point is the mean ± S.E.M. of 7 animals. \*\*P<0.01 different from control group. Significant differences (p<0.01) between animals pretreated with lithium for 14 and 28 days have been shown with (a).

Table 2. Effects of different doses of gentamicin on apomorphine-induced licking in animals pretreated with lithium for 14 and 28 days

Time (days)	Licks/60 min			
	Saline (2 µl/rat)	Gentamicin (10 µg/rat)	Gentamicin (20 µg/rat)	Gentamicin (40 µg/rat)
Control	1163±184	47±22	61±38	105±22
Li 14 days	1795±84*** <sup>a</sup>	41±23	55±21 <sup>b</sup>	73±28
Li 28 days	464±118**	319±69*	426±67*	116±48

Animals were injected either with saline (2 µl/rat, i.c.v.) or gentamicin (10, 20 and 40 µg/rat, i.c.v.) immediately before apomorphine (0.25 mg/kg, S.C.). \*P< 0.05 and \*\* p<0.01 different from control group. Significant differences between animals pretreated with LiCl (600 mg/l in drinking water) for 14 and 28 days have been showed with (a, p<0.01) and (b, p<0.05).

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