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# *IN VIVO* AND *IN VITRO* EFFECTS OF TETRAHYDROISOQUINOLINES AND OTHER ALKALOIDS ON RAT PITUITARY FUNCTION*

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**Abstract**—Several tetrahydroisoquinolines (TIQs) were tested for their *in vitro* and *in vivo* capacities to modulate prolactin (PRL) and β-endorphin (β-end) secretion by the rat pituitary and for their abilities to displace [$^3$H]spiroperidol and [$^3$H]naloxone binding from pituitary and hypothalamic membranes. Receptor binding studies showed that TIQs could be classified as having (a) higher affinity for opiate receptors (tetrahydropapaverine, papaverine, 6-methylsalsolinol, 1-carboxysalsolinol and 3′,4′-deoxy-norlaudanosolinecarboxylic acid), (b) higher affinity for the dopamine receptor (salsolinol and 7-methylsalsolinol), or (c) approximately equal affinity for the two binding sites (6,7-dimethylsalsolinol and tetrahydropapaveroline, THP). In freely moving male rats, THP produced a several-fold increase in plasma PRL levels. This effect was not altered by co-administration of naloxone but was attenuated by dopamine. *In vitro* several TIQs reversed the inhibitory effect of dopamine on PRL secretion by cultured anterior pituitary cells. The order of potencies of the TIQs in this system paralleled their order of potencies in the dopamine receptor assay. THP, the most potent dopamine antagonist, also blocked dopamine-mediated inhibiton of β-endorphin secretion from neurointermediate lobe cells in culture. These data demonstrate that THP and some other TIQs can act as dopamine antagonists in radioreceptor assays, in cell culture and *in vivo*.

Tetrahydroisoquinolines (TIQs) can be formed as condensation products of catecholamines and aldehydes by the Pictet–Spengler reaction. TIQs attracted increased attention following the demonstration by Davis and Walsh [1] that the alcohol metabolite acetaldehyde promoted conversion of [$^{14}$C]dopamine to [$^{14}$C]tetrahydropapaveroline (THP) *in vitro* and the suggestion that opioid activity of such metabolites might account for the addiction liability of alcohol. TIQs have since been identified in urine of Parkinsonian patients being treated with *l*-dopa [2] and patients with phenylketonuria [3]. Additionally, salsolinol, a TIQ of dopamine origin, has been found in brains of rats treated with pyrogallol and ethanol [4], in urine of non-pathologic human volunteers and at higher concentration in urine of intoxicated alcoholics [5].

There is increasing evidence that, if such TIQs are formed *in vivo* in sufficient quantities, they could interact with neurotransmitter systems in a variety of ways. Salsolinol and tetrahydropapaveroline bind to brain opiate receptors *in vitro* and produce analgesia *in vivo* [6] and have opiate-like effects in the guinea pig ileum bioassay [7]. Norlaundanosoline-carboxylic acid (NLCA) binds to opiate receptors and acts as an opiate agonist in decreasing serum luteinizing hormone (LH) levels following subcutaneous injection [8]. Another TIQ, 3-carboxysalsolinol, produces naloxone reversible analgesia in mice [9]. There is also evidence for interactions of TIQs with catecholamine systems. Nimitkitpaisan and Skolnick [10] demonstrated that THP, salsolinol and salsoline all interact with varying affinities with β-adrenergic receptors, inhibiting binding of [$^3$H]dihydroalprenolol and inhibiting isoproterenol stimulated accumulation of cAMP in brain slices. Beta-adrenergic blocking effects have also been demonstrated for 1-substituted TIQs in the perfused rabbit heart [11]. TIQs have similarly been shown to interact with dopaminergic systemes. THP acts as an antagonist to dopamine receptors in the molluscan gut [12] while salsolinol acts as a dopamine receptor agonist in that system.

We have studied the effects of a variety of TIQs (Fig. 1) in systems sensitive to dopamine and/or opiates in an effort to correlate *in vitro* effects with *in vivo* effects.

## MATERIALS AND METHODS

*Dopamine receptor assay.* Bovine anterior pituitaries were homogenized in 0.32 sucrose (20 vol.) using a Brinkman polytron and were centrifuged at 1,000 *g* for 10 min. The supernatant fraction was then recentrifuged at 48,000 *g* for 30 min. The resulting pellet was resuspended in 20 vol. (based on original

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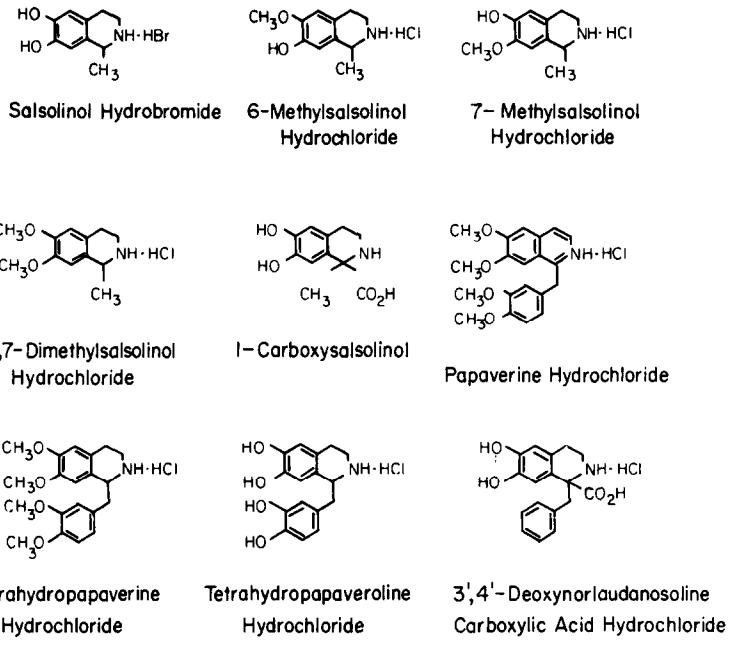


Fig. 1. Structures of tetrahydroisoquinolines (TIQs).

weight) of 50 mM Tris–HCl buffer, (pH 7.4) containing 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 120 mM NaCl, 10 $\mu$M pargyline, and 0.1% ascorbic acid. Tissue homogenates (400 $\mu$l) were incubated for 30 min at room temperature in the presence of 50 $\mu$l of unlabeled ligand followed by addition of 50 $\mu$l [$^3$H]spiroperidol and an additional 30-min incubation. At the end of the second incubation, 5 ml of ice-cold buffer was added to the tubes and the contents were filtered through Whatman GF-B filters. This was followed by a second rinse of the filtered tissue with 5.0 ml buffer. The filters containing the tissue and bound [$^3$H]spiroperidol were then dried, added to scintillation vials with 10 ml Aquasol (New England Nuclear Corp., Boston, MA), and counted in a liquid scintillation counter. The potencies of dopamine and the TIQs were established by their abilities to block 50% of the specific binding of 1.0 nM [$^3$H]spiroperidol. These potencies are expressed as $K_i$ values where

$$K_i = \frac{\text{Concn of TIQ producing 50\% inhibition}}{1 + (1.0\,\text{nM}/K_D)}$$

*Opiate receptor assay.* The opiate receptor assay was performed as described above with the following exceptions. [$^3$H]Naloxone (sp. act. 8.23 Ci/mmole) was used as the radioactive ligand. The $K_D$ of naloxone binding was 1.0 nM as established using 100 $\mu$M unlabeled naloxone to block specific binding. Rat hypothalami were prepared as described above for pituitary membranes, and incubations were for 30 min in an ice bath.

Initial screening of receptor binding activity was assessed by using TIQs at a concentration of 100 $\mu$M to block [$^3$H]spiroperidol or [$^3$H]naloxone binding. The resulting inhibition was expressed as percent inhibition of total binding.

*Cell cultures.* Anterior and neurointermediate, plus posterior, pituitary glands were removed from male Sprague–Dawley albino rats, separated, and prepared in tissue culture as previously described [13]. Quartered pituitaries were dissociated in 4-(2-hydropyethyl)-1-piperazine-ethanesulfonic acid (Hepes) buffer containing 3% bovine serum albumin, 0.1% hyaluronidase and 0.35% collagenase at 37° followed by washing and a 15–30 min incubation in 0.25% Viokase (Gibco). Dispersed cells were then centrifuged and resuspended in Dulbecco modified Eagles medium (DMEM) containing 10% horse serum, 2.5% fetal calf serum, 0.1 mM glutamine, 1.0% Gibco non-essential amino acids, 100 $\mu$g/ml ascorbic acid, and 5 $\mu$g/ml pargyline. Cells were repeatedly washed in this medium and then placed in culture dishes and removed to an incubator (37°) with an $H_2O$ saturated atmosphere of 10% $CO_2$, 90% air. Cells were incubated for 3 days to allow adhesion to the dishes. They were then washed four times in DMEM (without serum). Test solutions were added in volumes of 100 $\mu$l and triplicate dishes of cells were incubated for an additional 3 hr at which time the medium was removed, centrifuged, diluted with 0.9% NaCl containing 1% bovine serum albumin (BSA) and stored frozen at −20° for later radioimmunoassay. Prolactin (PRL) was assayed using a radioimmunoassay kit provided by NIAMDD. $\beta$-Endorphin ($\beta$-end) was assayed by radioimmunoassay using antibody (provided by Dr. Alberto Panerai) which is directed towards the Leu$^{14}$-His$^{27}$ region of $\beta$-end. This antibody cross-reacts with ovine $\beta$-lipotrophin ($\beta$-LPH) (100%) but to only a minor extent (<0.1%) with Met$^5$ or Leu$^5$ enkephalin.

In vivo *effects on prolactin release.* Male Sprague–Dawley albino rats weighing about 300 g were implanted with chronic venous catheters in the jugular vein. The implanted tubing was threaded

under the skin to exit at the base of the skull. After a period of 3–4 days recovery, animals were placed in individual cages. Drugs were given, and blood samples were taken via a tube attached to the catheter. Blood samples were taken immediately before, and 5 and 15 min after, injection of the drugs. Control animals received an equal volume of 0.9% NaCl. After centrifugation, plasma samples were assayed for prolactin.

*Drugs.* The following drugs were purchased from commercial sources: dopamine (Sigma Chemical Co., St. Louis, MO), haloperidol (Haldol®, McNeil Laboratories, Fort Washington, PA), naloxone (Narcan®, Endo Laboratories, Garder City, NY), papaverine HCl (Sigma Chemical Co.), and 6,7-dimethylsalsolinol HCl (ICN-K & K Laboratories, Inc. Plainview, NY). Salsolinol HBr, 6-methylsalsolinol HCl, 7-methylsalsolinol HCl, 1-carboxysalsolinol, and 3′,4′-deoxynorlaudanosolinecarboxylic acid HCl (DNLA) were synthesized at the Salk Institute Alcohol Research Center by Pictet–Spengler condensations [14] between dopamine or methylated derivatives (Sigma Chemical Co.) and the appropriate aldehyde or ketone. Tetrahydropapaverine HCl and tetrahydropapaveroline HCl (THP) were prepared by the method of Pyman [15] except that THP was recrystallized from 6 N HCl by the method of Teitel *et al.* [16]. All compounds exhibited the expected melting points. Tritiated naloxone and tritiated spiroperidol were purchased from the New England Nuclear Corp.

## RESULTS

*Receptor binding studies.* In initial screening, several TIQs showed an ability to inhibit the binding of [$^3$H]naloxone and/or [$^3$H]spiroperidol to rat hypothalamic or bovine anterior pituitary tissue respectively. The effects of the various TIQs at concentrations of 100 $\mu$M are shown in Table 1. The greatest effects on [$^3$H]naloxone binding were produced by tetrahydropapaverine > papaverine = DNLA > 6-methylsalsolinol ≃ salsolinol ≃ 1-carboxysalsolinol ≃ THP > 7-methylsalsolinol ≃ 6,7-dimethylsalsolinol. Inhibition of [$^3$H]spiroperidol binding followed the order of: THP > salsolinol > 7-methylsalsolinol ≃ 7-methylsalsolinol ≃ tetrahydropapaverine > papaverine > 6-methylsalsolinol ≃ 6,7-dimethylsalsolinol > DNLA ≃ 1-carboxysalsolinol. The more potent of these TIQs were selected for further study to establish $K_i$ values versus the two $^3$H-ligands where

$$K_I = \frac{IC_{50}}{1 + \frac{L}{K_D}}$$

These values, representing the mean of two to four assays, are reported in Table 2. Given the fact that naloxone interacts with multiple classes of opiate receptors, the $K_i$ for a displacing ligand may not precisely reflect the affinity of that ligand for any single receptor sub-type. Based upon these results

Table 1. Effects of TIQs on binding of [$^3$H]naloxone and [$^3$H]spiroperidol*

| Tetrahydroisoquinoline (100 $\mu$M) | % Inhibitor [$^3$H]Naloxone binding to rat hypothalamus | % Inhibitor [$^3$H]Spiroperidol binding to bovine anterior pituitary |
|---|---|---|
| Salsolinol HBr | 44 | 48 |
| 6-Methylsalsolinol HCl | 47 | 19 |
| 7-Methylsalsolinol HCl | 25 | 39 |
| 6,7-Dimethylsalsolinol HCl | 24 | 16 |
| 1-Carboxysalsolinol | 46 | 0 |
| Papaverine HCl | 52 | 30 |
| Tetrahydropapaverine HCl | 61 | 36 |
| Tetrahydropapaveroline HCl | 45 | 57 |
| 3′,4′-Deoxynorlaudanosolinecarboxylic acid HCl | 52 | 4 |

* Values are the means of two to three determinations done in duplicate. There was less than a 10% difference among replicates.

Table 2. Potencies of TIQs in inhibiting [$^3$H]spiroperidol or [$^3$H]naloxone binding*

| TIQ | $K_i$ ($\mu$M) vs [$^3$H]spiroperidol | $K_i$ ($\mu$M) vs [$^3$H]naloxone |
|---|---|---|
| Salsolinol | 11.1 ± 4.2 | — |
| 7-Methylsalsolinol | 12.0 ± 5.6 | — |
| Papaverine | 94.3 ± 24.3 | 23.0 ± 5.1 |
| Tetrahydropapaverine | 7.8 ± 0.5 | 13.0 ± 2.8 |
| Tetrahydropapaveroline | 2.8 ± 0.7 | 20.0 ± 4.3 |
| Deoxynorlaudanosolinecarboxylic acid | — | 20.0 ± 8.3 |

* Values are the means ± S.E.M. of two to four determinations done in duplicate.

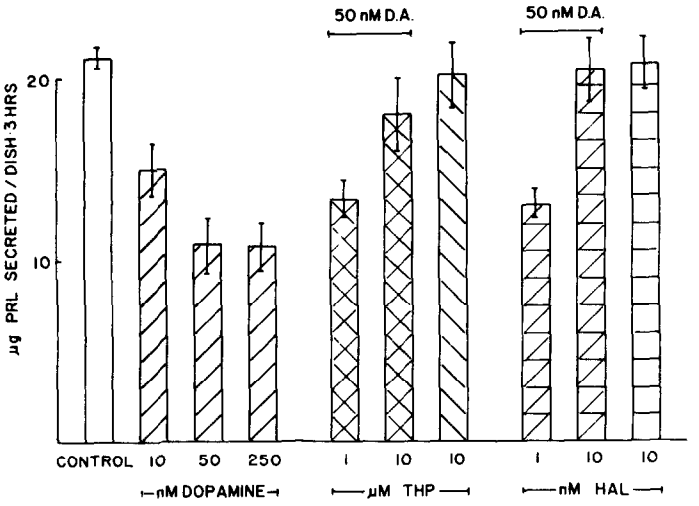


Fig. 2. Effects of tetrahydropapaveroline (THP) and haloperidol (HAL) alone and in combination with dopamine (DA) on prolactin (PRL) secretion from cultured anterior pituitary cells. Values are means ± S.E.M.

some of the TIQs were selected for *in vivo* and *in vitro* tests which could demonstrate either dopaminergic or opioid actions.

Effects of TIQs on immunoreactive prolactin (PRL) and β-end secretion from cultured anterior and neurointermediate lobe pituitary cells were studied. Prolactin secretion by cultured anterior pituitary cells was inhibited by dopamine. This inhibition was blocked by the dopamine receptor blocker haloperidol and, as shown in Fig. 2, was likewise blocked in a dose-dependent manner by THP. Neither THP nor haloperidol had any effect on PRL secretion in the absence of dopamine. Figure 3 shows the effects of salsolinol, 7-methysalsolinol, 6,7-dimethylsalsolinol and tetrahydropaverine on dopamine-inhibited PRL secretion. In a dose range of 1.0 to 25.0 μM salsolinol, 7-methylsalsolinol and tetrahydropapaverine showed effects similar to those of THP in antagonizing the inhibitory affects of dopamine.

The secretion of β-end from cultured rat neurointermediate lobe cells was also under direct inhibitory control of dopamine. In another experiment it was shown that 50 nM dopamine almost totally inhibited the β-end-like immunoreactivity secreted by these cells (Fig. 4). THP (1, 5 or 25 μM) antagonized this inhibition with the antagonism being essentially complete at a THP concentration of 5.0 μM. As with the anterior pituitary cells, the secretory rate for the neurointermediate lobe cells was not affected by THP in the absence of dopamine.

In vivo *effects of THP and DNLA on PRL secretion.* Two TIQs were selected to study their effects

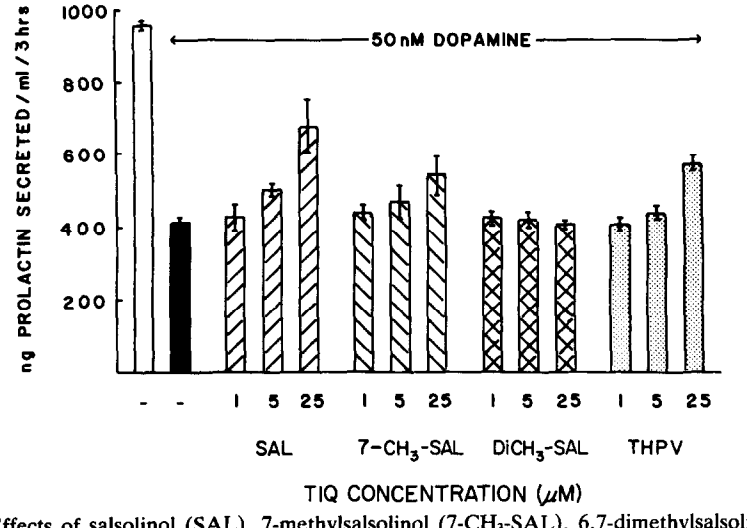


Fig. 3. Effects of salsolinol (SAL), 7-methylsalsolinol (7-$CH_3$-SAL), 6,7-dimethylsalsolinol (Di$CH_3$-SAL) and tetrahydropapaverine (THPV) on dopamine inhibition of PRL secretion by cultured anterior pituitary cells. Values are means ± S.E.M.

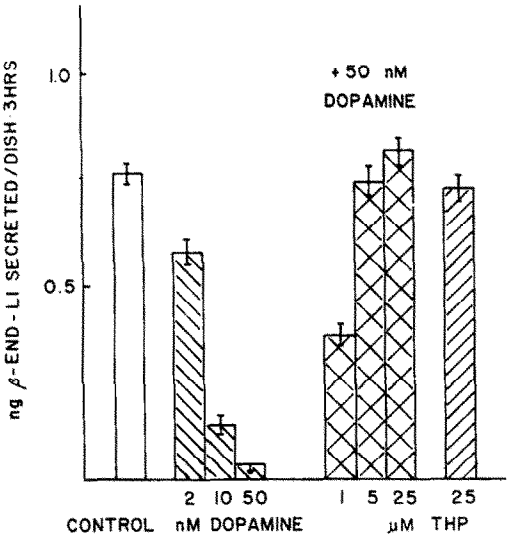


Fig. 4. Effects of dopamine and THP alone and in combination on beta-endorphin-like immunoreactivity (β-end-LI) secretion by cultured posterior neurointermediate pituitary cells. Values are means ± S.E.M.

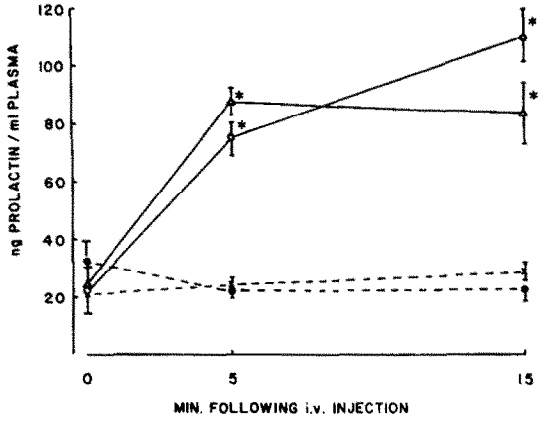


Fig. 5. Effects of THP (3.0 mg/kg) (○——○) and naloxone (1.5 mg/kg) (·-----·) alone and in combination (△——△) on plasma PRL levels following i.v. injection. Saline control (×------×). Each point is the mean ± S.E.M. of five animals. Key: (*) significantly different from saline control ($P < 0.01$).

on plasma PRL in freely moving male rats. Plasma samples were taken immediately before and 5 and 15 min following injection of drugs. THP, which showed affinity for both the opiate and the dopamine receptor sites, was injected at a dose of 3.0 mg/kg, i.v. As shown in Figs. 5 and 6, THP produced a substantial rise in plasma PRL. Figure 5 demonstrates that the co-administration of the opiate antagonist naloxone slightly decreased basal and THP-stimulated PRL levels but had no significant effect on either. Figure 6 shows the interaction of THP with dopamine (0.3 mg/kg). The co-administration of dopamine significantly attenuated the effects of THP. The TIQ with the greatest selectivity for the opiate over the dopamine receptor was

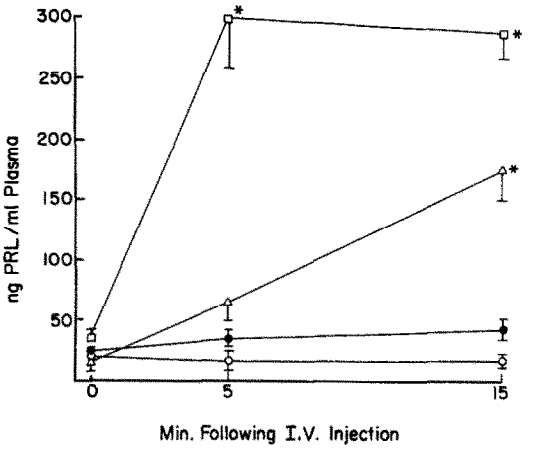


Fig. 6. Effects of THP (□——□) (3.0 mg/kg, i.v.) and dopamine (○——○) (0.3 mg/kg, i.v.) alone and in combination (△——△) on plasma levels. Saline control (●——●). Each point is the mean ± S.E.M. of five animals. Key: (*) significantly different from saline control ($P < 0.01$).

DNLA. Figure 7 shows that, while haloperidol also increased PRL secretion, neither DNLA nor naloxone alone or in combination interfered with this effect.

## DISCUSSION

Previous reports demonstrated the formation of TIQs from catecholamines and acetaldehyde *in vitro* [1, 17]. More recent findings indicate their presence following *l*-dopa administration in rat brain [18] and human urine [2]. Collins *et al.* [5] have recently shown salsolinol to be present in the urine of normal human volunteers and to a greater extent in the urine of alcoholic volunteers at the start of detoxification. Lasala and Coscia [3] showed that children with

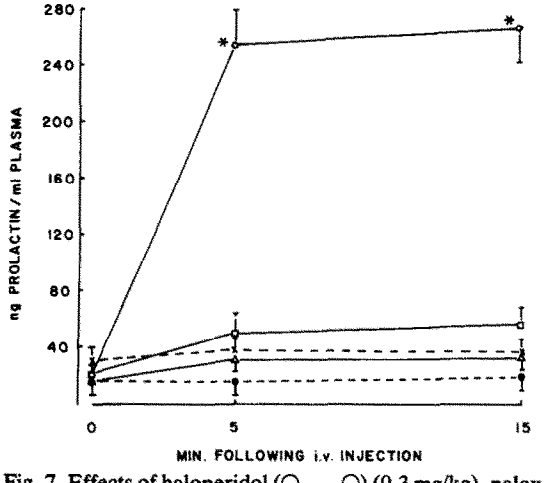


Fig. 7. Effects of haloperidol (○——○) (0.3 mg/kg), naloxone (●---●) (1.5 mg/kg), DNLA (△——△) (3.0 mg/kg) and DNLA + naloxone (□——□) on plasma PRL levels. Saline control (×------×). Each point is the mean ± S.E.M. of five animals. Key: (*) significantly different from saline control ($P < 0.01$).

phenylketonuria had measurable quantities of DNLA in their urine. These results demonstrate *in vivo* and support the hypothesis that under some conditions TIQ formation could reach significant proportions contributing to disease or pharmacologic processes.

One of the featurs of TIQs which has emerged is their seeming ability to interact in a variety of systems. Due to their catecholamine origin and their structural similarities to certain opioids, most research has focused on TIQ interactions with various processes related to these two classes of putative neurotransmitter systems. The ability of TIQs to exhibit opiate-like effects has been shown in several systems. Analgesic effects have been demonstrated in laboratory animals for 3-carboxysalsolinol [9] and for salsolinol and THP [6]. Salsolinol has also been shown to have opiate agonist effects in the guinea pig ileum bioassay [7]. DNLA was shown by Lasala *et al.* [8] to have opiate agonist-like effects in the receptor binding assay and in decreasing serum LH levels *in vivo*.

The $K_i$ values for TIQs in displacing [$^3$H]naloxone were evaluated in a buffer system containing 120 nM $Na^+$, thus favoring the binding of antagonists [19]. In fact, Lasala *et al.* [8] have shown a considerable sodium shift effect for DNLA indicating, along with biological effects, that this compound acts as a relatively pure opiate agonist. The values reported here for papaverine, tetrahydropapaverine, THP and DNLA are very close in fact to those reported by Lasala *et al.* for DNLA in the presence of sodium. Opiates have been shown to increase PRL secretion *in vivo* but not in isolated pituitary cells [20]. This indicates an extrapituitary site of action. Additional support for this interpretation comes from the findings that beta-endorphin decreases dopamine turnover in the median eminence and also increases serum PRL levels [21] and that the opiate antagonist naltrexone blocks the PRL releasing effects of intrahypothalamic beta-endorphin [22]. In addition, naloxone has been shown to decrease basal and opiate stimulated plasma PRL levels [23]. Similar, though not significant, changes are reported here for naloxone effects on basal PRL levels.

In light of the prevailing evidence, we might have expected that THP or DNLA could alter PRL release by acting at opiate receptors located on hypothalamic dopaminergic neurons. Thus, opiate agonists would be expected to decrease the release of dopamine from these neurons resulting in a lessening of the inhibitory effects of dopamine on PRL release and a consequent increase in serum PRL. THP which has affinity for both dopamine and opiate receptors did result in increased serum PRL values. This effect was partially inhibited by co-administration of dopamine but was not affected by naloxone. DNLA which was much more selective in competing for the opiate receptor failed to have any significant effect on PRL secretion. On this basis we conclude that the demonstrable effect of THP and DNLA in opiate receptor binding studies is not apparent as an *in vivo* effect on PRL secretion at the dose tested. This is not inconsistent with the data of Lasala *et al.* [8] who found opiate-like effects of norlaudanosolinecarboxylic acid on serum LH levels only at doses of 7.0 mg/kg and higher. This TIQ has the same Na-dependent affinity for [$^3$H]naloxone binding sites as we report for DNLA.

The effects of several TIQs on the dopaminergic system are readily apparent. These effects can be demonstrated in competition for [$^3$H]spiroperidol binding sites in the pituitary. The receptor interaction can be demonstrated as one of antagonism to the $D_2$ (non-cyclase linked) receptor of the anterior pituitary by the ability of THP, salsolinol, 7-methylsalsolinol and tetrahydropapaverine to reverse the inhibitory effects of dopamine on PRL secretion by cultured anterior pituitary cells. The most potent of these (THP) was also shown to be effective in reversing the inhibitory effects of dopamine on beta-endorphin release from culture rat neurointermediate lobe cells. Finally, THP showed dopamine antagonist affects *in vivo* in increasing serum PRL in a fashion modulated by co-administration of dopamine but not naloxone. These data are also consistent with the demonstration by Dougan *et al.* [12] that THP blocks the inhibitory actions of dopamine in a molluscan gut preparation. It has been shown that THP and salsolinol bind to beta-adrenergic receptors as well as haloperidol binding sites and appear to act as partial agonists on the beta-receptor adenylate cyclase system of rat cerebral cortex [10]. There is also evidence that the posterior-neurointermediate lobe contains beta-adrenergic receptors and that both norepinephrine and phosphodiesterase inhibitors increase basal secretion of beta-endorphin from cultured NIL cells [24]. Our results show no effect of THP on basal secretion of beta-endorphin while there is a reversal of dopamine inhibition of secretion. These results suggest that in this system also the predominant effect of THP is to act as an antagonist at the dopamine receptor.

Interpretation of the physiological or pharmacological role of TIQs remains difficult in light of the low concentrations reported in normal or drug (i.e. *l*-dopa or ethanol)-treated subjects related to the concentrations required to produce biological effects thus far studied. The lack of knowledge regarding the metabolic half-life of these compounds and their microdistribution leaves open the possibility that they may be involved in normal physiology or in drug effects associated with *l*-dopa treatment or ethanol ingestion.

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