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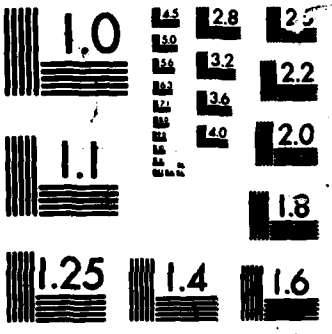

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MUSCARINIC ANTAGONISTS FREE OF HALLUCINOGENIC PROPERTIES

Annual Report

W.J. Rzeszotarski, L.J. Grimm, L.A. Rothblat

December 1984

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QNB (3-quinuclidinyl benzilate), a potent anti-muscarinic agent possessing strong central stimulatory/hallucinogenic properties, and two analogues, QNX (3-quinuclidinyl xanthene-9-carboxylate) and QNA (3-quinuclidinyl atrolactate) synthesized in our laboratory, were evaluated in the rat for their central effects on behavior.

In the pilocarpine test ~~(Fig. 1)~~, QNB gave a smooth dose-response curve with maximum interference of pilocarpine-induced catatonia at 5mg/Kg. The inhibition decreased in a dose-dependent manner to control levels of catatonia at 0.01 mg/Kg. QNX and QNA gave responses markedly different from QNB.

QNB, QNX and QNA were all potent stimulators of the limb flick response in rats. All three compounds, in their racemic forms have equal potency to produce limb flick ~~(Fig. 2)~~ at a level which compares to that seen after administration of LSD ~~(10)~~.

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The results may indicate a greater bioavailability of QNA of QNA when compared to QNB and QNX since the results obtained with QNA are remarkably similar to those seen with QNX in both paradigms and QNB in the limb flick response.

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The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH 78-23, Revised 1978).

SUMMARY

QNB (3-quinuclidinyl benzilate), a potent anti-muscarinic agent possessing strong central stimulatory/hallucinogenic properties, and two analogs, QNX (3-quinuclidinyl xanthene-9-carboxylate) and QNA (3-quinuclidinyl atrolactate) synthesized in our laboratory, were evaluated in the rat for their central effects on behavior. Two behavioral measures were selected: 1) The pilocarpine test, a measure of interference with pilocarpine induced catatonia thought to be centrally mediated by blockade of the muscarinic acetylcholine receptor in the nigro-striatum; and 2) The limb flick response, which is thought to be indicative of hallucinatory activity in the rat.

In the pilocarpine test (Fig. 1), QNB gave a smooth dose-response curve with maximum interference of pilocarpine-induced catatonia at 5 mg/Kg. The inhibition decreased in a dose-dependent manner to control levels of catatonia at 0.01 mg/Kg. QNX and QNA gave responses markedly different from QNB. Both compounds exhibited curves which were sharply bi-phasic; maximal inhibition at 5.0 mg/Kg decreased to produce control levels of catatonia at 0.5 mg/Kg, then inhibition increased again from 0.5 to 0.1 mg/Kg. In the dose range 0.1 to 0.01 mg/Kg, inhibition of the catatonic response again decreased to control levels.

QNB, QNX and QNA were all potent stimulators of the limb flick response in rats. All three compounds, in their racemic forms have equal potency to produce limb flick (Fig. 2) at a level which compares to that seen after administration of LSD (10). The rank order of potency in the limb flick response does not correspond to the rank order of potency of these compounds' ability to bind to the cholinergic muscarinic receptor subtyped M_1 which is QNX = QNB > QNA. QNX and QNA are selective for the M_1 receptor while QNB has approximately equal affinity for M_1- and M_2-receptors. QNA has an overall 10-fold lesser affinity for muscarinic receptors. This rank order of binding affinities is not seen in the limb flick response, nor in the pilocarpine response. Interestingly, the resolved R-isomer of QNB is significantly more potent than QNB (racemate or the S-isomer), QNX and QNA in producing both the limb flick response and inhibition of the pilocarpine response.

The first year's results may indicate a greater bioavailability of QNA when compared to QNB and QNX since the results obtained with QNA are remarkably similar to those seen with QNX in both paradigms and QNB in the limb flick response. An alternate hypothesis suggested by the data is that the hallucinatory activity of QNB and its analogs is unrelated to interaction with the muscarinic acetylcholine receptor in the brain and may be mediated by activity in another receptor system.

INTRODUCTION

Since the early work of Abood (1) it has been known that the glycolate class of anti-muscarinic compounds such as: benactyzine, ditran, and 3-quinuclidinyl-benzilate (QNB), possess strong central stimulatory preperties which effect behavior. The hallucinogenic and behavioral properties of the glycolate esters have been assumed to be caused by the compound's activity at the muscarinic acetylcholine receptor in the brain (Abood 1968).

Recent evidence from our laboratory (2) and others (3-6) indicates that the central muscarinic acetylcholine receptor may exist as two or more subtypes. This observation has led us to hypothesize that the anti-muscarinic (and potentially nerve agent protective) properties of QNB or its congeners might be separable from the hallucinatory properties on the basis of interaction at the different subtypes of the muscarinic receptor, designated M_1 and M_2.

We have demonstrated by radioligand binding assay that two compounds synthesized in our laboratory, QNYT (3-quinuclidinyl xanthene 9-carboxylate) and QNA (3-quinuclidinyl atrolactate) have a selective affinity for the M_1 and M_2 receptors. Further, QNX has equal or greater affinity for muscarinic receptors than QNB while QNA demonstrates a ten-fold lesser affinity for the receptors. This selectivity for the M_1 receptor and the range of overall affinity for the muscarinic receptor provides a useful tool to explore the behavioral effects of these compounds.

Two behavioral paradigms were employed to attempt to establish a rank-order of potency for QNB, QNX, and QNA in the test which measure effects on centrally mediated behavior. The first behavioral model is the pilocarpine test. It has been demonstrated that pilocarpine induces a centrally mediated catatonia, presumably by stimulating muscarinic acetylcholine receptors in the nigro-striatum (7,8). We employed the test to determine the central anti-muscarinic activity of QNB and analogues by measuring interference with pilocarpine induced catatonia. It was assumed that antagonism by QNB, QNX, and QNA at the M_1 or M_2 receptors would produce interference with catatonia in a dose-dependent manner with the rank order of potency being determined by: 1) which muscarinic receptor (M_1 or M_2) mediates the pilocarpine responses; 2) the order of affinity for binding to the M_1 receptors; and 3) the relative rates of absorption of the three compounds into the brain from an intraperitoneal (i.p.) injection site.

The second behavior measure, the limb flick response, is an easily quantifiable, robust, unconditioned behavior seen in rats and cats (9) following the adminstration of a hallucinogen like LSD. There is some question about the specificity of the behavior (10) since it was learned that a non-hallucinogen (in man), methysergide, also caused limb flicking behavior. The behavioral effects of hallucinogen were recently the subject of an excellent review (11). This

review indicated that while it is not known how tightly the limb flick response is coupled to known hallucinatory activity in man, evidence suggests that a connection does exist and therefore the response can be a reliable, quick measure of potential hallucinatory activity. Abood (1) has seen limb flicking after administration of QNB and it was therefore assumed that this behavior associated with QNB could also be linked to its known hallucinatory activity in man.

As a preliminary working hypothesis, it was assumed that the M_1 selective compounds QNX and QNA would be shifted to the left of QNB in the pilocarpine test, indicating increased potency as a central anti-muscarinic. Further, these M_1 selective compounds would be shifted to the right of QNB in the limb flick response, indicating a decreased ability to cause limb flick and therefore assumed to have a decreased hallucinatory potential.

METHODS

Animals

248 male Sprague-Dawley rats in the weight range of 170-300 g were used to perform the pilocarpine test. 240 mal Sprague-Dawley rats in the weight range of 170-340 g were used for the limb flick response. All rats were housed, 5 to a cage, in hanging wire mesh cages for at least one week prior to use. The rats were given tap water and rat chow ad libitum and maintained on a 12 hour light cycle (0700-1900 hours). All experiments were performed between 0900 and 1500 hours in well-lighted, quiet rooms.

Compounds

All compounds were given i.p. at a volume of 1ml/Kg except methyl atropine nitrate which was 0.5ml/Kg. QNB, QNX and QNA were dissolved in a small amount of DMSO (0.5-1.0%) and taken to volume with 5mM tartrate buffered saline immediately prior to dosing. Methyl atropine nitrate and pilocarpine HCl were dissolved in saline immediately prior to use. All compounds were made fresh just prior to injection and no solutions were used more than once due to the limited solubility of these compounds and to the fact that their stability properties in solution were unknown.

Dosages

In the pilocarpine test doses of QNB, QNX and QNA were given to ten rats per compounds per dose with dises ranging from 0.01mg/Kg to 10 mg/Kg. At least six dose levels were tested per compound, with additional doses added to clarify the results previously obtained. Methyl atropine nitrate was given at a dose of 0.25mg/Kg and pilocarpine at 100 mg/Kg.

Each animal was only tested once due to the persistance of QNB in the brain, which has been demonstrated previously (15).

In the limb flick response, 10 rats were treatd per dose of QNB (racemate), R-QNB, QNX or QNA in doses ranging from 0.1 to 20 mg/Kg. The dose and compound were randomized for time of day, day of the week and the week tested, with dosing completed in a cycle of five weeks.

Behavioral Measures

Pilocarpine-Induced Catatonia. Five to ten rats were pretreated with QNB, QNX or QNA at a given dose to which had been added methyl atropine nitrate. Thirty minutes after pretreatment each rat was injected the opposite side of the midline with pilocarpine. At ten minute intervals post-pilocarpine, the rats were placed

on a seven cm high horizontal bar with the fore limbs draped over the bar and the hind limbs providing standing support. One observer placed the rats on the bar and the other began the stopwatch count when the first observer's hand left the animals. Catatonia time was defined as the time it took the animal to remove both paws from the bar. Two trials were performed for each ten minute interval and the testing period lasted for sixty minutes post-pilocarpine administration.

Limb Flick Respnse. Two rats were dosed with the same compound and identical dose and placed side by side in a plastic, rectangular cage with opaque sides and a clear front and back. The cage was tall enough so that the rats could not climb out or see each other. The rats did not undergo a pre-dose acclimitization and the environment was, therefore, novel to them at a dosing. The rats' movements were videotaped in order to have a record to review, however, in most cases the animals were observed directly for limb flicking behavior. The number of limb flicks produced by each rat was counted by an observer and recorded at five minute intervals. Limb flicking behavior was defined as a vigorous shaking of one or both of the fore-paws. Other behaviors seen such as head shakes, body shakes, unusual gaits, etc. were also noted. Control animals which had been treated with vehicle were also observed.

RESULTS AND CONCLUSIONS

Pilocarpine-Induced Catatonia

As seen in figure 1, the catatonia time (the time each rat remained on the bar averaged over the sizty minute testing period) is plotted against the dose received for QNB, QNX and QNA. Analysis of variance of catatonia time vs. dose for the 3 compounds indicates there is a significant interaction ($F_{8, 135}=5.57$, p .005) between compound and dose for QNB, QNX, and QNA in the pilocarpine test.

QNB When tested for the ability to interfere with pilocarpine-induced catatonia, QNB produced a smooth dose response curve between 0.01 and 5.0 mg/Kg (Fig 1). The ID_{50} is estimated to be 0.075 mg/Kg. Two way ANOVA shows a significant relationship between dose and response ($F_{4, 135}=3.91$, $p < .005$) and between compounds ($F_{2, 135}=6.58$, $p < .005$). QNB at 5.0 mg/Kg produces maximal inhibition (catatonia scores of 0) and a dose of 0.01 mg/Kg catatonia has returned to control levels (65 seconds/test period). The dose response curve for QNB lies to the left of QNX and QNA, and ANOVA indicates that the QNB response is significantly different from that of QNX and QNA.

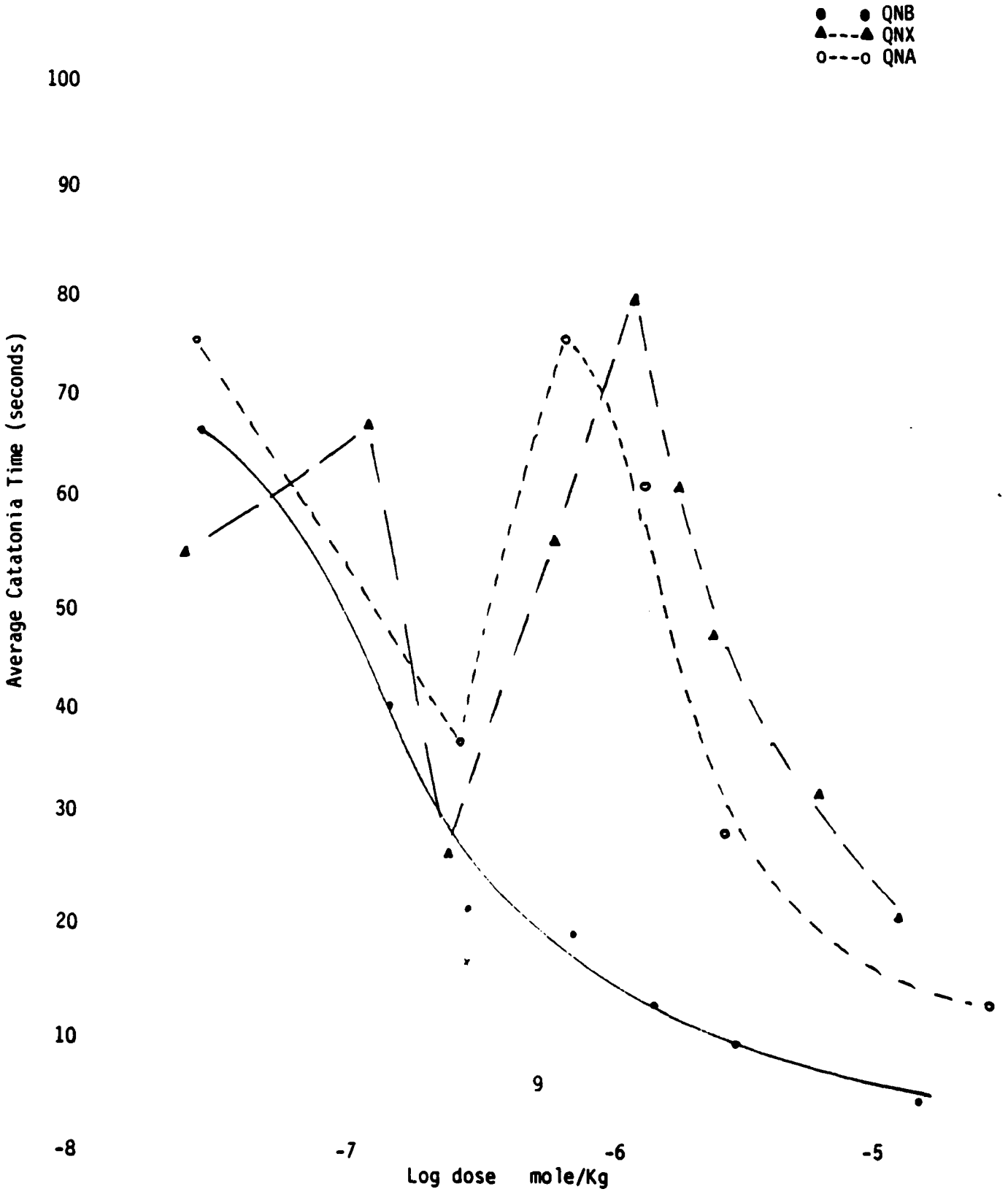
QNX QNX interfered with pilocarpine-induced catatonia in a much different manner than did QNB. The dose response curve for QNX, over a similar dose range to QNB, is sharply bi-phasic (Fig 1). An ID_{50} would be difficult to determine since it appears that there i disinhibition of catatonia occurring between 0.1 and 0.5 mg/Kg. At the lowest doses tested, 0.01 to 0.1 mg./Kg, QNX produces a response similar to QNB. However, at 0.25 and 0.5 mg/Kg an increase in catatonia time is seen, a response which could be likened to a disinhibition of the inhibitory effect seen at lower doses. At doses ranging from 0.75 to 5.0 mg/Kg, interference with pilocarpine-induced catatonia is once again seen to occur in a dose-dependent manner. Perhaps it is noteworthy that the maximal inhibition of catatonia seen with QNX is at a dose somewhat higher than that seen with QNB, and catatonia scores of zero were not seen even at the highest dose of QNX tested, 5.0 mg/Kg.

QNA QNA interferes with the catatonic response in a manner quite similar to QNX (Fig. 1). The dose-response curve for QNA overlays that for QNX with a slight shift to the left. It may be quite possible that maximal catatonia occurs at a dose which lies between 0.5 and 0.25 mg/Kg. QNA exhibits the same sharply bi-phasic curve that was seen with QNX and ANOVA indicates that QNX and QNA are significantly different from QNB, but a t-test indicates that QNX and QNA are not significantly different from each other. It is most likely that the "disinhibition" seen is the result of the compounds' effect at another, non-muscarinic receptor system which could be interfering with the results obtained. Costall and Naylor (7) have pointed out that catatonia is produced by the nigro-striatum via stimulation of the acetylcholine receptor (pilocarpine response) or by blockade of the dopamine receptor. Recent evidence indicates

FIGURE 1

INHIBITION OF PILOCARPINE RESPONSE AFTER ADMINISTRATION OF QNB, QNX, AND QNA

CATATONIA TIME VS. LOG DOSE



that the two systems may in fact be quite closely connected and that the dopamine system may rely on the ACh system to produce its effects (12). It seems unlikely that our data can be due to erratic absorption since halfo of each dose group is obtained on different days with new drug solution, but the results obtained for the two groups are always consistent.

An interesting correlate to the pilocarpine data has been suggested by Trulson and Crisp (13) who point out that the effect of LSD on dopamine receptors is that of a mixed agonist-antagonist and that some combination of serotonergic-dopaminergic activity by LSD may be required for the production of hallucinations.

Limb Flick Response

The frequency of limb flicks in the 45 minute testing period is plotted against dose of the QNB (racemate), R-QNB, QNX and QNA as seen in Figure 2. QNB, QNX, and QNA, in their racemic forms, have equal potency to produce limb flick in the rat. ANOVA demonstrated no significance with respect to a compound by dose interaction ($F_{8, 135}=1.75$, $p<.1$) indicating that the dose response curves of QNB, QNX, and QNA are not significantly different. All three compounds exhibited similar dose response curves in the range of 0.1 to 5.0 mg/Kg. At dose higher than 5.0 mg/Kg, general responding was abnormal and so the response fell off (Fig. 2). The resolved R-isomer of QNB is significantly ($F_{12,180} = 3.02$, p .005) more potent than QNB (racemate or S-isomer), QNX or QNA in producing the limb flick repsonse. The dose response in the limb flick did not reflect the rank order of binding affinities previously stated nor did the response resemble the rank order seen in the pilocarpine test.

Other behaviors which were noted but not quantitated include: head and/or body shaking, aborted grooming episodes which were usually associated with the limb flick, and hind limb "tapping" which may have resembled limb flicking if the hind limbs were not used for support. All of these behaviors have been associated with administration of hallucinogens such as LSD and further have been noted after administration of high doses of atropine (14).

If the limb flick response and therefore the hallucinatory potential of QNB and its analogues is unrelated to interaction at the muscarinic acetylcholine receptor in the brain then the compounds would not necessarily follow the rank order of binding affinity nor of the pilocarpine response, which is known to be mediated by the muscarinic acetylcholine receptor in the brain.

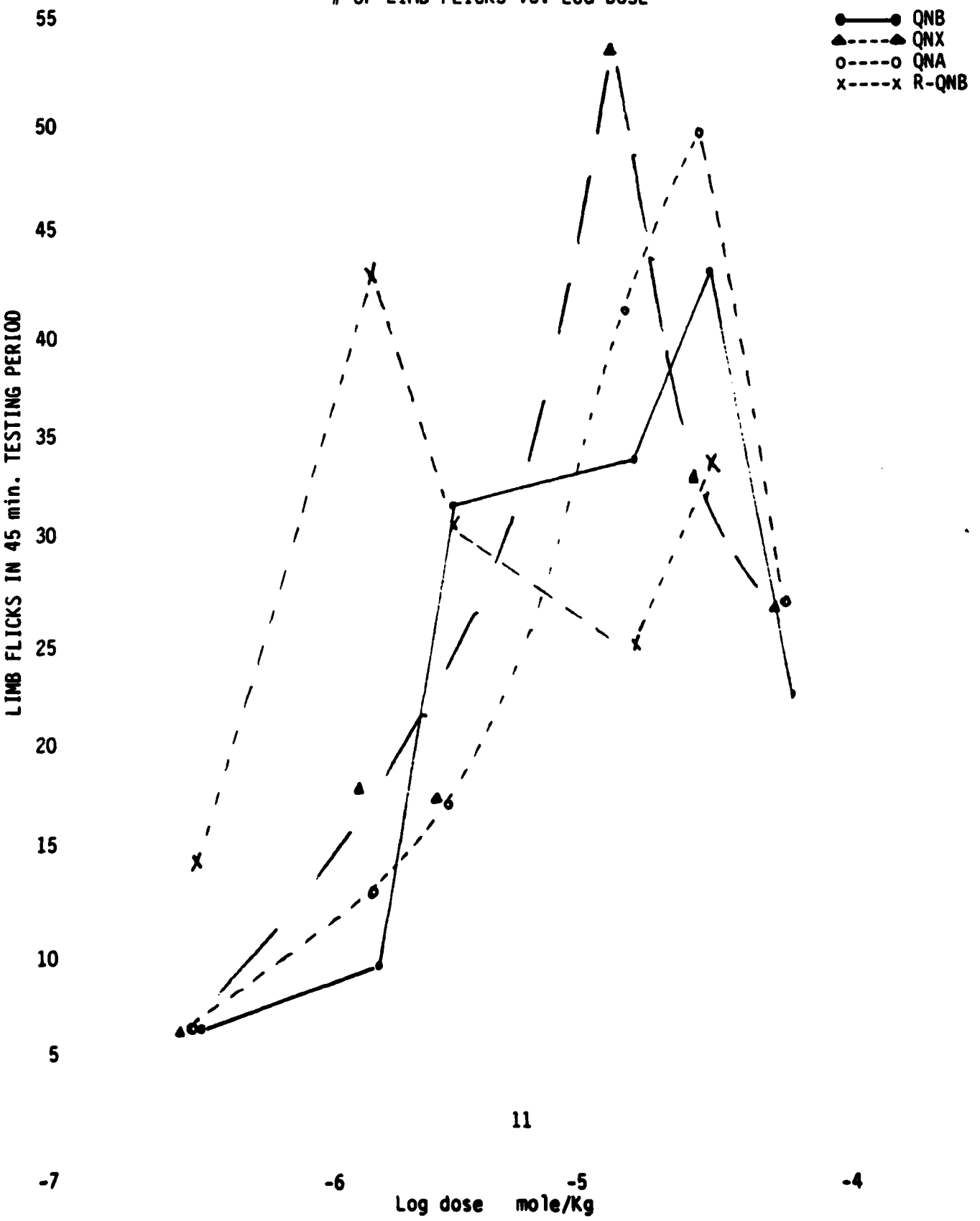
If QNA has a greater bioavailability than QNB and QNX it might be possible for QNA to overcome the decrease in potency of binding affinity to the muscarinic acetylcholine receptor and produce a response equal to QNB and QNX. This does not explain the pilocarpine test results, however, where QNA is equipotent with QNX, but less than potent than QNB.

The data seem instead to argue that some activities of these compounds may be mediated through another receptor system to produce the limb flick and cause interference with the pilocarpine response. The most likely choice of receptor at which these compounds may be exhibiting additional effects is the serotonergic receptor. Most of the hallucinogens which have been shown to produce limb flick have been subsequently shown to be potent serotonergic agonists (11).

FIGURE 2

LIMG FLICK RESPONSE AFTER ADMINISTRATION OF QNB, R-QNB, QNX, and QNA

OF LIMB FLICKS VS. LOG DOSE



Future Work

Work is already in progress to evaluate the binding affinities of the resolved isomers of QNB, QNX, and QNA in the serotonergic and dopaminergic receptor systems in the brain. Synthesis and behavioral evaluation of the stereisomers of other QNB analogues has also begun. Studies to evaluate the central behavioral effects of these compounds on cholinergic systems which impact on learning are being planned and should be implemented within four months. The overall thrust of the pharmacological research will be to continue to evaluate the anti-cholinergic activity of the analogues on centrally-mediated behavior and to explore the possibilities that the undesirable hallucinatory effects of QNB may be mediated by an interaction which mimics that of LSD, and is non-muscarinic in nature. It is though that the global effects of LSD may be brought about by a complex interaction at serotonin receptors and by an agonist-antagonist action at dopamine receptors. We have preliminary evidence that QNB and analogues may have an action at these other receptors. Future work will attempt to clarify the role that QNB and its analogues may be playing at the serotonin and dopamine receptors to provide insight toward design of new compounds with the aim of eliminating the "hallucinatory component" of these potent anti-muscarinics.

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