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Effects of Physostigmine on the Startle in Guinea Pigs: Two Mechanisms Involved

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PHILIPPENS, I. H. C. H. M., B. OLIVIER AND B. P. C. MELCHERS. *Effects of physostigmine on the startle in guinea pigs: Two mechanisms involved.* PHARMACOL BIOCHEM BEHAV **58**(4) 909–913, 1997.—The effects of the acetylcholinesterase inhibitor physostigmine (PHY) on the auditory startle reflex in guinea pigs were studied. The dose–response curve of PHY appeared bell shaped, with a maximum effect dose of 0.3 mg/kg. In addition, PHY altered the shape of the startle response. The muscarinic antagonist scopolamine (SCO) increased the startle at PHY doses above 0.3 mg/kg without affecting the PHY-induced shape of the response. The decreasing part of the startle due to PHY could be mimicked by the cholinesterase inhibitor soman in combination with 0.3 mg/kg PHY. It appeared that the decreasing part of the dose–response curve at higher dose levels is caused by the cholinesterase inhibitory action of PHY and, in view of the SCO effect, is mediated by muscarinergic receptors. The increasing part of the curve is probably caused by an agonistic action of PHY on neuronal nicotinergic receptors, because the antagonist mecamylamine (20 mg/kg) antagonized the effects of 0.3 mg/kg PHY both on the deflection and shape of the startle. © 1997 Elsevier Science Inc.

Physostigmine Auditory startle reflex Cholinergic system Guinea pig

THE acoustic startle response in rodents is a sensitive method to determine how different neurotransmitter systems modulate sensorimotor activity (5). The role of the cholinergic system in modulating the startle reflex is far from clear. No consistent effects of cholinergic drugs on the startle response have been reported (8,9,12,19). These studies therefore support the conclusion of Davis (5), that the cholinergic system only plays a small and indirect role in the startle response. However, in a study (14) on behavioral side effects of the combination of the acetylcholinesterase inhibitor physostigmine (PHY) and the muscarinic antagonist scopolamine (SCO), we noticed unexpected effects on the startle reflex. Although some behavioral and neurophysiological side effects, caused by PHY, could be antagonized by a low dose of SCO, the addition of SCO considerably enhanced, and not antagonized, the increase of the startle response. It is unlikely that the effects of PHY on the startle response are caused by its acetylcholinesterase (AChE)-inhibiting effect because these effects have not been reported for other AChE inhibitors. Diisopropyl fluorophosphate slightly enhanced the startle reflex (5), while another organophosphate AChE inhibitor, soman, slightly decreased it (unpublished data). Para-n-phenylphosphoramidate, a reversible organophosphate AChE inhibitor (11), alone or in combination with SCO, had no effect on the startle, although AChE inhibition in the brain was similar as found after PHY administration (14). This indicates that besides AChE inhibition additional effects of PHY may be involved in its effects on the startle response.

Such effects of PHY, unrelated to AChE-inhibition, have been reported earlier (2,4,16). PHY may have both agonistic and antagonistic effects on nicotinergic ACh receptors (2,4,16). In the present study an explanation is given for the previous reported effects of PHY, SCO, and their combination (14) on the startle reflex.

METHOD

Animals

Male Dunkin–Hartley albino guinea pigs CrL:(HA)BR (Charles River) with an initial body weight of 350–400 g were used. Three animals were kept in one cage (Makrolon type IV). The ambient temperature was regulated between 20–

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22°C. Relative humidity was monitored but not regulated and was over 50%. Food and water were always available. An independent ethical committee advised positively on the described experiments.

General Procedure

To obtain control values, the startle response was measured in all animals 1 day before drug injections. Subsequently, on the basis of the obtained results, comparable subgroups were formed of five or six animals each. Thereafter, the animals were subcutaneously injected with the drugs under investigation. Startle responses were measured 30 min and 24 h after injection. Only those animals with predrug startle responses of more than 100 g were used in analysing of the drug effects.

Auditory Startle Response

The animals were exposed to 20 auditory startle pulses while standing in a vertically mounted PVC tube (diameter 7 cm, length 16.5 cm), resting with their hind paws on a platform. The startle-eliciting stimulus consisted of a 20 ms, 120 dB, 10 kHz bandpass filtered burst of white noise. Startle responses were measured by a transducer connected with the platform. For the duration of 100 ms and in later experiments 200 ms the force exerted by the hind paws upon presentation of the stimulus was registered. In this way only the startle response of the hind paws was recorded. The data were digitized (50 Hz) by the ADC of an IBM compatible personal computer, averaged and stored on disk for later analysis. The area under the curve (AUC) measured for the duration of 100 ms after presentation of the startle pulse was used to quantitate the startle reflex. Only in the last experiment we measured the AUC during 200 ms.

Statistics

An analysis of variance (ANOVA) followed by a Newman–Keuls post hoc test was used to assess statistical significance. In case pre- and postdrug values were compared in one animal the Wilcoxon matched pairs signed-rank test was used. p-Values < 0.05 were considered significant.

Drugs

Physostigmine (eserine) and scopolamine bromide were obtained from Sigma, St. Louis, MO; Mecamylamine hydrochloride was obtained from Merck Sharp & Dohme International, Rahway, NJ; Soman (O-pinacolyl methylphosphonofluoridate) was synthesized at the Prins Maurits Laboratory TNO (Dr. H. P. Benschop).

RESULTS

The PHY dose–response curve (DRC) on the startle reflex is shown in Fig. 1. It shows a bellshaped response curve; the maximal effective dose of PHY being around 0.3 mg/kg, $F(4, 18) = 4.67$, $p = 0.0092$. The effect of PHY is not only an effect on the amplitude of the startle. At all doses used, PHY induces a change of the shape of the startle response (Fig. 2). After having reached the maximal amplitude, the curve did not return to baseline within the 100 ms registration time, but showed a "shoulder." When longer registration periods (200 ms) were applied it lasted about 140 ms before the startle response returned to baseline (Fig. 6).

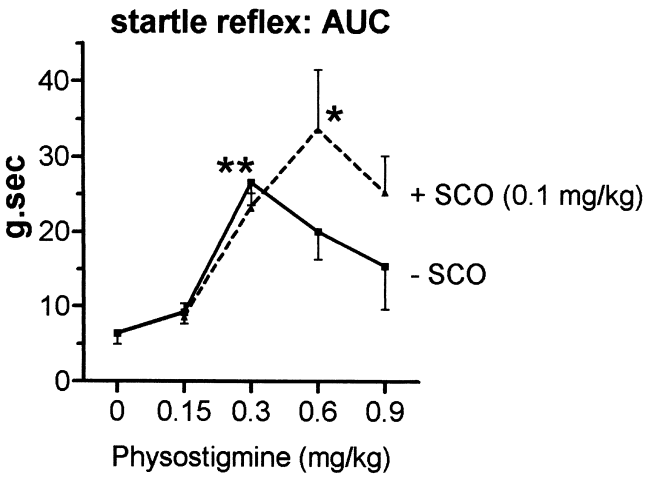

FIG. 1. Mean (±SEM) of the AUC of the startle response of 100-ms duration (startle pulse: 20 ms, 120 dB, 10 kHz). Registration of the effects 30 min after SC injection of saline, PHY (0.15, 0.3, 0.6, or 0.9 mg/kg), or PHY (0.15, 0.3, 0.6, or 0.9 mg/kg) + SCO (0.1 mg/kg), n = 5 or 6 animals/group. *Significantly different, between PHY and PHY/SCO-treated groups, using analysis of variance and Newman–Keuls post hoc test $p < 0.05$. *Significantly different, between PHY and control value, using analysis of variance and Newman–Keuls post hoc test $p < 0.05$.

SCO (0.1 mg/kg) combined with different doses of PHY (Fig. 1) only had an increasing effect at doses of PHY higher than the maximal effective dose, but did not affect the "shoulder" in the response curve, $F(1, 8) = 7.16$, $p = 0.028$. The percentual changes induced by SCO (0.1 mg/kg) at the two higher PHY dose levels, compared to PHY, were significantly increased, $F(3, 18) = 5.66$, $p = 0.0065$. This curve also appeared to be bell shaped. The decrease of the startle reflex at the highest dose of PHY compared with the combination of SCO with PHY (0.6 mg/kg) was not caused by an incomplete antagonistic activity of SCO. The SCO dose tested (0.1 mg/

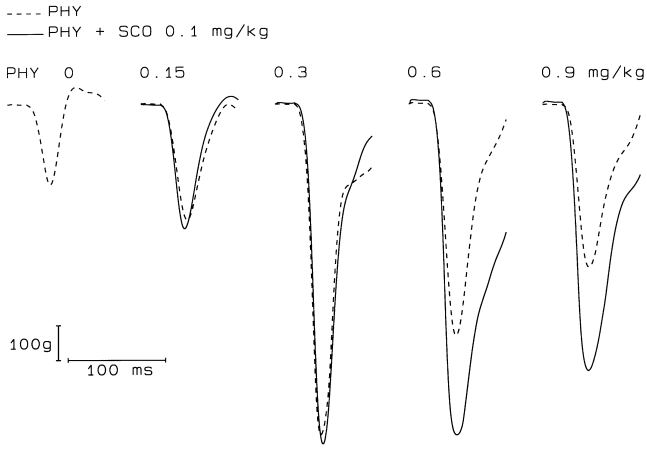

FIG. 2. Mean of the startle response of 100 ms duration (startle pulse: 20 ms, 120 dB, 10 kHz). Registration of the effects 30 min after SC injection of: PHY (0, 0.15, 0.3, 0.6, or 0.9 mg/kg): dotted lines, or PHY (0.15, 0.3, 0.6, or 0.9 mg/kg) + SCO (0.1 mg/kg): bold lines, n = 5 or 6 animals/group.

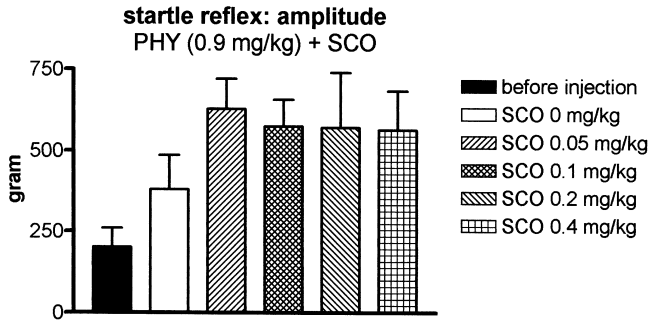

FIG. 3. Mean (±SEM) of the amplitude of the startle response of 100-ms duration (startle pulse: 20 ms, 120 dB, 10 kHz). Registration of the effects 30 min after SC injection of saline, PHY (0.9 mg/kg) + SCO (0.05, 0.1, 0.2, or 0.4 mg/kg), $n = 6$ animals/group.

kg) appeared to be already maximally effective; higher and lower doses of SCO did not result in a significantly larger or smaller enhancement of the startle (Fig. 3), $F(4, 22) = 0.64$, $p = 0.64$.

These drugs did not affect general motor activity: the number of intertrial responses, a measurement of the activity level, obtained in an active avoidance task, showed no differences between before and after injection (SCO 0.1 mg/kg, $F(1, 12) = 1.87$, $p = 0.196$; PHY 0.6 mg/kg, $F(1, 14) = 2.18$, $p = 0.1616$, or 1.2 mg/kg, $F(1, 14) = 0.49$, $p = 0.4935$ (13).

To find out whether the effect of PHY at higher dose levels was caused by the AChE inhibitory capacity of PHY, we tried to mimick the descending second phase of the DRC of PHY by applying low doses of the AChE inhibitor soman combined with 0.3 mg/kg PHY. In Fig. 4 the DRC of PHY is compared with the curve composed of the maximal effect dose of PHY and PHY in combination with two different dosages of soman (0.3 and 0.6 × LD_{50} (=0.025 mg/kg) (7). These curves appeared similar, $F(2, 15) = 1.96$, $p = 0.17$. However, soman did not affect the shape of the startle response after PHY. A single administration of soman at the highest dose (0.015 mg/kg SC) resulted in a small but insignificant decrease of the startle

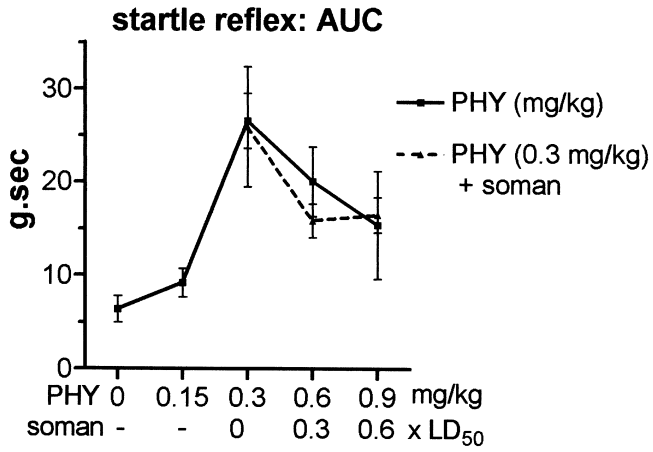

FIG. 4. Mean (±SEM) of the AUC of the startle response of 100-ms duration (startle pulse: 20 ms, 120 dB, 10 kHz). Registration of the effects 30 min after SC injection of PHY (0.3 mg/kg) + Soman (0xLD_{50} or 0.3xLD_{50} or 0.6xLD_{50}), $n = 6$ animals/group, compaired with the DRC of PHY. The LD_{50} dose of soman is 0.025 mg/kg SC (6).

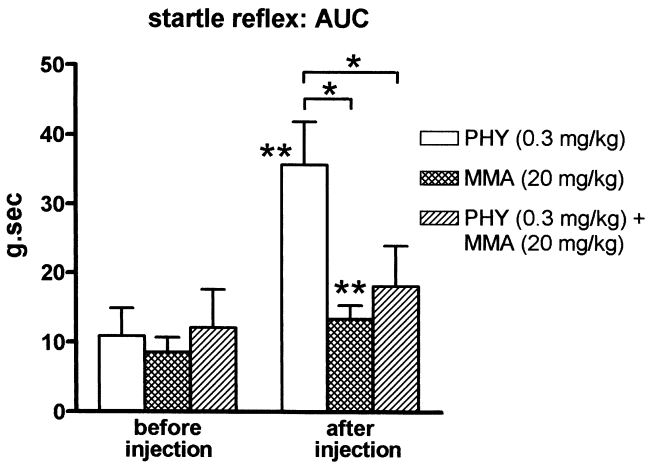

FIG. 5. Mean (±SEM) of the AUC of the startle response of 200-ms duration (startle pulse: 20 ms, 120 dB, 10 kHz). Registration of the effects 30 min after SC injection of Mecamylamine (MMA) (20 mg/kg), PHY (0.3 mg/kg), or MMA (20 mg/kg) + PHY (0.3 mg/kg), $n = 6$ animals/group. *Significantly different using analysis of variance and Newman–Keuls post hoc test $p < 0.05$. *Significantly different using Wilcoxon matched-pairs signed-ranked test $p < 0.05$.

response compared to a control group before and 30 min after injection, $F(3, 44) = 2.04$, $p = 0.1223$.

To investigate the involvement of nicotinergic receptors, the effect of the neuronal nicotinergic receptor antagonist mecamylamine (MMA) was tested in combination with the maximal effect dose of PHY. MMA at a dose of 20 mg/kg SC caused a small but significant increase of the startle compared with the preinjection value ($p = 0.05$, Wilcoxon matched pairs signed-rank test). MMA (20 mg/kg) completely antagonized the effect of PHY (0.3 mg/kg): the startle response after the combination of MMA and PHY was significantly smaller than the startle found after PHY (0.3 mg/kg) alone (Fig. 5), $F(2, 15) = 5.41$, $p = 0.017$. Furthermore, the typical "shoulder" in the startle response always present after PHY administration, also disappeared when PHY was given in combination with MMA (Fig. 6).

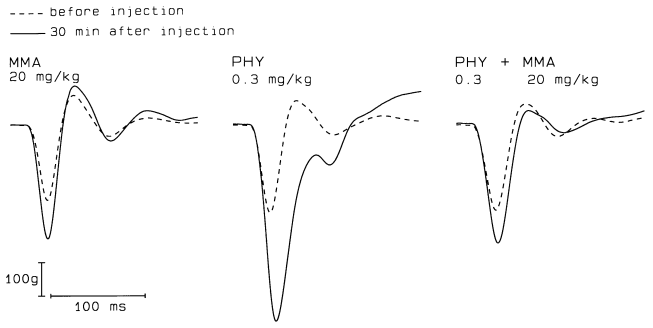

FIG. 6. Mean of the startle response of 200 ms duration (startle pulse: 20 ms, 120 dB, 10 kHz). Registration of the effects after SC injections of Mecamylamine (MMA) (20 mg/kg), PHY (0.3 mg/kg), or MMA (20 mg/kg) + PHY (0.3 mg/kg), before injection: dotted lines, or 30 min after injection: bold lines, $n = 6$ animals/group.

DISCUSSION

In this study the pharmacology of the effects of PHY on the auditory startle reflex of the guinea pig were investigated. According to Davis (5), ACh only plays a minor or indirect role in the startle modulation. This view seems justified when the effects on the startle response of different types of cholinesterase inhibitors are considered: only small effects were reported (5,14). However, our present data disagree with this opinion. We demonstrated a clear enhancing effect of PHY on the startle response, especially when PHY was combined with SCO. This cannot be due to general motor effects because previously it was demonstrated (see the Results section) that the motor activity was not influenced.

As argued before, it is unlikely that this enhancing effect of PHY can be ascribed to inhibition of AChE, because other cholinesterase inhibitors have failed to induce such an effect, even at doses leading to larger levels of brain AChE inhibition (14). Therefore, additional effects of PHY may be involved. Several effects of PHY, other than inhibition of AChE have been described. PHY may act both as an agonist and an antagonist on nicotinergic receptors (2,4,16). Interestingly, the ED_{50} of PHYs' agonism at the nicotinergic receptor appears to be lower than its IC_{50} of AChE inhibition (3). In view of the antagonism of MMA, a neuronal nicotinergic receptor antagonist, on the PHY effects on the startle, our results may be explained by an agonistic action of PHY on these receptors. This is in agreement with the results of Acri et al. (1), who have shown that nicotine causes a dose-dependent increase of the startle response. This effect of PHY apparently occurs already at very low PHY concentrations in the brain. A change of the shape of the startle response, showing a characteristic shoulder, was seen even at the lowest PHY dose we used (0.15 mg/kg) (Fig. 2). The slight increase of the startle found after MMA might be due to stress factors caused by the injection.

Interestingly, it appeared that when the AChE-inhibition in the brain reaches a certain level, the extra stimulation of cholinergic receptors leads to a decrease of the startle response that was increased by low doses of PHY. Therefore, it appears that PHY antagonizes its own effect on the startle response. This latter effect of PHY can be mimicked by giving another AChE inhibitor soman and may be antagonized by the muscarinic antagonist SCO. SCO, at doses of 0.1, 0.2, or 0.4 mg/kg, had no effect on the startle response (14). Activation of this inhibitory cholinergic system leads to a decrease of the startle. However, in view of the lack of effect of other AChE inhibitors on the startle (5,14), this system is only effective when the startle is increased following PHY administration.

This is corroborated by others: PHY, at higher dose levels (i.e., via AChE inhibition), activates neurons inhibiting the primary startle pathway. It appears that the pedunculopontine tegmental nucleus (PPTg) plays an important role in modulating sensorimotor gating by linking the ventral pallidum and the nucleus reticularis pontis caudalis, an obligatory part of the primary startle (6,17,18), via a direct, presumably muscarinic, cholinergic projection (10). This inhibitory circuit can be activated by acetylcholine agonists (10). Furthermore, lesions of the PPTg lead to an increased startle amplitude (18).

However, not all effects of PHY appear to be antagonized by AChE inhibition; the characteristic shoulder in the startle response remains present at all dose levels of PHY tested. This shoulder is responsible for the fact that the startle response curve found after PHY does not reach the baseline within 100 ms. Normally, the response curve reaches the baseline within 100 ms, which is seen in the control responses. This could lead to an underestimation of the effects established.

The nicotinergic receptor antagonist MMA was the only drug in this experiment that could also antagonize the shape of the startle curve.

On the basis of our results it is not possible to decide whether the nicotinic actions of PHY directly affect the startle or that other transmitter systems are involved. It has, for example, been shown that nicotine may enhance the release of 5-HT (15), and it has been shown that one of the major transmitter systems involved in the startle reflex is the serotonergic system (5).

In conclusion, the results demonstrate that PHY affects the startle response by two different but coupled mechanisms: a startle activating mechanism that is most likely due to an agonistic action of PHY on nicotinergic receptors, and another a startle inhibiting mechanism that is most likely due to activation of muscarinergic receptors that are triggered after activation of the nicotinergic system.

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