

2-PAM extracellularly to the neuron-neuron synapse in the brain of a subject by administering Pro-2-PAM to the subject.

Additional Pro-2-PAM Experiments

[0100] Additional experiments showed that 1 min post-exposure treatment with Pro-2-PAM also protected against multiple LD₅₀ of cutaneous soman exposure. In the above guinea pig skin model, OP compounds penetrate across the skin and then enter the systemic circulation. Animals were weighed and their lateral sides clipped using an electric clipper with a #40 blade one day before the experiment. On the day of the study, animals had a central 3×4 cm area marked using a permanent marker as the site for OP application, i.m. pyridostigmine (0.026 mg/kg) 30 min prior to OP exposure and then an i.m. injection (in a rear leg) using the combination of ketamine (32 mg/kg) and xylazine (4 mg/kg) 5 min prior to OP exposure. Guinea pigs in individual cages were placed in the chemical fume hood, and the OP compound was applied in a droplet, to the center of the marked area. Typical soman volume varied between 0.05 and 500 μl. Exactly 1 min post-exposure, guinea pigs were given atropine sulfate (16 mg/kg) in one leg i.m. and in the other 2-PAM or Pro-2-PAM in the other leg. Animals were observed continuously for the first 4 hr after exposure and intermittently for the next 4 hr or to the end of the work day. Signs of soman intoxication and the time of onset of each were recorded. Animals were evaluated at 24 hr post-exposure, and surviving animals were euthanized by injecting 75 mg/kg pentobarbital followed by terminal cardiac puncture exsanguination. Brain, blood, and diaphragm tissues were frozen on dry-ice for ChE assays. In this guinea pig model, the LD₅₀ for soman with saline (control) treatment yielded 11.3 mg/kg, while 3 autoinjector equivalents of 2-PAM (25.7 mg/kg) increased the LD₅₀ to 66.7 mg/kg soman. This indicates the PR (protective ratio) of 2-PAM is about 5.9, a significant improvement in survivability of the animals. Treatment with equivalent Pro-2-PAM provided an LD₅₀ of 119 mg/kg soman, and a PR of about 10.5, which is almost twice (i.e. 1.8×) that of 2-PAM. All survivability curves were sigmoidal in shape. Thus, Pro-2-PAM exhibited better protection than 2-PAM (10.5 vs 5.9, respectively). Additionally, H&E stained images of the hippocampal neuron layer of soman exposed (45 mg/kg) and 2-PAM treated animals exhibited marked disruption of this layer, swollen neurons, and indistinct nuclei, in contrast to soman exposed (91 mg/kg) and Pro-2-PAM treated animals which exhibited an intact hippocampal layer. Unexpectedly, in contrast to that observed in the art, demonstrate: (1) the importance of a stable composition of Pro-2-PAM, (2) neuroprotection, and (3) rapid biotransformation to 2-PAM in the CNS and increase survivability against cutaneous OP exposure.

[0101] Therefore, the present invention provides methods for increasing the survivability of a subject after cutaneous exposure to an OP compound, which comprises administering the subject Pro-2-PAM in a therapeutically effective amount.

+HUPA and PRO-2-PAM

[0102] As disclosed herein, the beneficial effects of +HupA in treating OP induced SE causing seizures, SE and neuropathogenesis were discovered to be due to its NMDA antagonist activity in the EAA excitatory pathway after OP exposure rather than its activity as a ChE inhibitor. Also, as disclosed herein, it was discovered that Pro-2-PAM was effective in

treating OP induced SE causing seizures, SE and neuropathogenesis, and that its protective mechanism is likely due to its ability to reactivate AChE in brain and reduce the buildup of ACh.

[0103] Because +HupA and Pro-2-PAM exhibit different protective mechanisms against seizures, SE causing seizures and SE caused by exposure to OP compounds, in some embodiments, the present invention is directed to combination treatments and compositions comprising both +HupA and Pro-2-PAM. Therefore, in some embodiments, the present invention provides methods for treating, preventing, inhibiting, or reducing seizures, SE causing seizures, SE and/or neuropathogenesis caused by exposure to an OP compound which comprises administering to a subject in need thereof a therapeutically effective amount of Pro-2-PAM and a therapeutically effective amount of +HupA. In these embodiments, Pro-2-PAM and +HupA may be administered at the same or different times before, during, or after OP exposure or a combination thereof. For example, +HupA without Pro-2-PAM may be administered prior to exposure to an OP compound, then after exposure, both Pro-2-PAM and +HupA may be administered at the same time.

Other Therapeutic Observations

[0104] +HupA. It was found that animals exposed to NMDA after pre-treatment with 3 mg/kg +HupA showed normal physical activity and that the quiet period observed following NMDA exposure was completely eliminated in +HupA treated animals. Behavior of animals pre-treated with 3 mg/kg +HupA was very similar to normal rats by visual observation. +HupA pre-treatment also maintained a normal heart rate of about 300-500 bpm. The body temperature of rats pre-treated with +HupA tended to be very unstable, but remained within the normal temperature range throughout the 24 hr monitoring period. The physical activity of animals was not affected by post-NMDA exposure treatment with 3 mg/kg +HupA. It was also discovered that +HupA is devoid of the side-effects such as behavior decrements which are normally associated with NMDA ion-channel antagonists. Rats treated with post-exposure +HupA showed normal baseline body temperature throughout the 24 hr recording period. These experiments show that +HupA does not have any observable cardiovascular toxicity. Therefore, +HupA may be administered to subjects with little or no observable cardiovascular toxicity.

[0105] Administration of Pro-2-PAM exhibited additional therapeutic advantages. For instance, the parameters of heart rate (BPM), body temperature (T, ° C.), and physical activity (counts/min) were recorded for 24 hr exposure to OP compounds results in prolonged hypothermia, bradycardia, and decreased activity due to fasciculation and fatigue, all of which remained depressed for at least 24 hr after exposure. See Gordon et al. (1996) *Pharmacol Biochem Behav* 55(2): 185-94. Treatment with 2-PAM partially modulated these responses, e.g. a long lag phase was observed before body temperature returned to normal. However, Pro-2-PAM treatment abrogated DFP induced hypothermia and bradycardia and restored activity. Therefore, the present invention also provides methods of treating, preventing, inhibiting or reducing hypothermia and bradycardia and reduced activity caused by exposure to an OP compound.

Delivery Methods

[0106] Pro-2-PAM and a huperzine A compound, e.g. +HupA, may be administered to a subject using methods,